International Journal of Advanced Research in Science, Engineering and Technology

ISSN: 2350-0328

Vol. 12, Special Issue, December 2025

National Conference on Earth, Elements and Energy: Interdisciplinary Perspectives (NC3EIP–2025)

Kinetic Study of Nitration of Acetanilide Using Spectrophotometric Analysis

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ABSTRACT: The kinetics of acetanilide nitration were investigated using UV–Visible spectrophotometric analysis to elucidate the reaction mechanism and rate dependence on various parameters. The reaction was carried out using a nitrating mixture of concentrated nitric and sulfuric acids, while the progress of nitration was monitored by measuring the absorbance of the reaction mixture at characteristic wavelengths. The influence of acetanilide concentration, nitrating mixture ratio, solvent polarity, and temperature on the reaction rate was systematically examined. The kinetic data indicated first-order dependence with respect to acetanilide concentration. Temperature variation studies followed the Arrhenius relationship, allowing determination of the activation energy. The results demonstrated that solvent polarity and acid composition markedly affected the rate constant. The study establishes a reliable spectrophotometric method for kinetic monitoring and provides valuable insights into the mechanistic aspects of electrophilic aromatic substitution reactions involving acetanilide.

KEY WORDS: Nitration, Kinetics, Acetanilide, Spectrophotometry, Reaction rate

I. INTRODUCTION

Nitration of aromatic compounds represents one of the central transformations of organic chemistry and remains one of the most broadly used electrophilic aromatic substitution reactions. Nitroaromatic compounds are important intermediates in the preparation of pharmaceuticals, dyes, agrochemicals, and energetic materials, so that their pathways of formation represent a key point of interest for academic and industrial chemistry. Among the various substrates investigated, acetanilide holds a particular position since its acetamido group acts as a director for incoming electrophiles mainly toward the para-position, at the same time exerting measurable inductive effects on the ring. Its reactivity is predictable; thus, it represents an ideal model substrate for mechanistic and kinetic studies. Traditional analysis in aromatic nitrations is essentially through titrimetric or product-based analytical techniques, which, besides being largely devoid of real-time resolution, may also mask the subtlety in kinetic variations. Spectrophotometric techniques, in turn, allow continuous monitoring of the course of reaction with more accurate determinations of rate behavior under controlled conditions. While nitration in mixed acid systems is understood in broad mechanistic terms, systematic spectrophotometric kinetic studies of acetanilide-which examines the concerted effects of temperature, solvent environment, nitrating mixture composition, and substituent effects on reaction rate-are limited. A dataset that unifies these interdependent variables is essentially missing in the literature. The present study addresses this gap by applying UV-Visible spectrophotometry to monitor the nitration of acetanilide in real time and quantify how key experimental parameters affect the reaction kinetics. The specific objectives are: *(1) to establish kinetic order and the rate constants for nitration of acetanilide under controlled conditions; (2) To determine the influence of nitrating acid composition, solvent polarity and structural substituents on rate of reaction; (3) to investigate temperature dependence and obtain activation energy with the Arrhenius relationship; and (4) to establish a robust spectrophotometric protocol that can serve as a reliable methodology for studying electrophilic aromatic substitution reactions in related systems*.

II. MATERIALS AND METHOD

A. Chemicals and Reagents

All chemicals used were of analytical reagent grade. Acetanilide (AR grade) was obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). Concentrated nitric acid (HNO_3, 69%) and concentrated sulfuric acid (H_2SO_4, 98%) were procured from Merck. Glacial acetic acid, ethanol, and distilled water were used as solvents without further purification. All solutions were freshly prepared before use to ensure reproducibility of results.

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B. Instrumentation

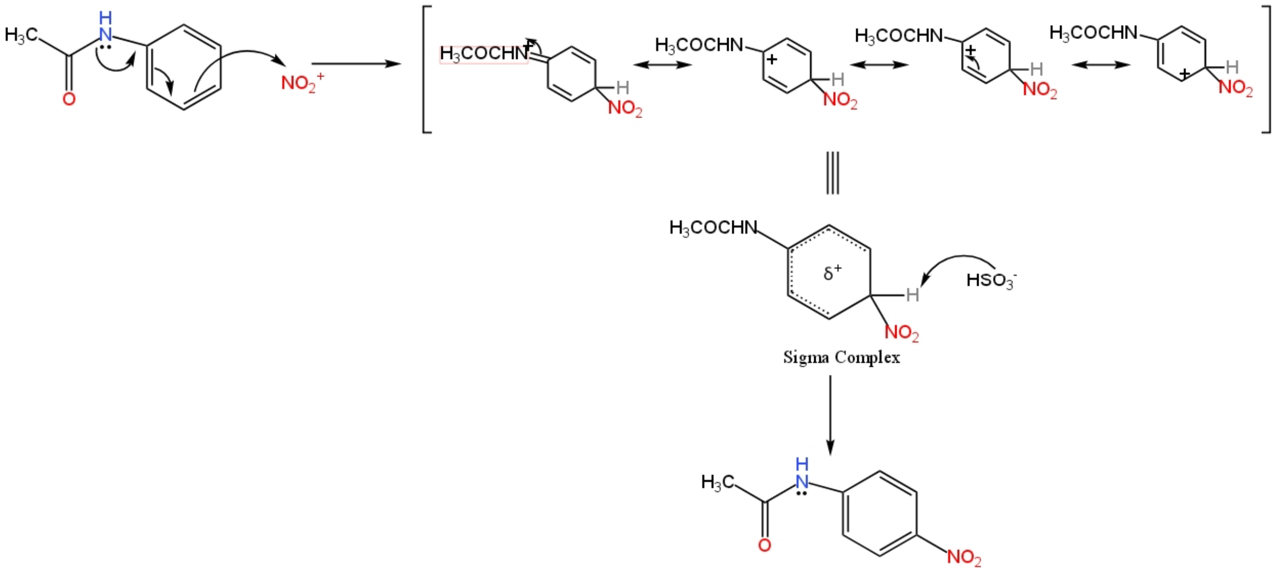
Spectrophotometric measurements were performed on a Shimadzu UV-1800 double-beam UV-Visible spectrophotometer and Jasco UV 770 spectrophotometer using 1 cm matched quartz cuvettes. Temperature control during kinetic runs was maintained using a thermostatic water bath with an accuracy of ±0.5 ◦C. All experiments were conducted under ambient laboratory light to avoid photo- chemical interference.

C. Preparation of Nitrating Mixture and Solutions

The nitrating mixture was prepared by slowly adding concentrated nitric acid to concentrated sulfuric acid in a specified volume ratio (typically 1:1 by volume) with continuous stirring and external cooling, following established procedures for generating the nitronium ion in mixed-acid systems [1, 2]. Stock solutions of acetanilide were prepared in glacial acetic acid and diluted as required to obtain different concentrations, consistent with earlier kinetic investigations employing acetic acid as a controlled reaction medium [5, 8].

D. Experimental Procedure

In a typical kinetic run, a pre-measured volume of acetanilide solution was equilibrated to the desired temperature alongside the nitrating mixture to ensure uniform thermal conditions. The reaction was initiated by rapidly combining equal volumes of the acetanilide solution and the freshly prepared nitrating mixture under constant stirring. Immediate and efficient mixing was essential to ensure consistent generation of the nitronium ion and reproducible reaction initiation. Immediately after mixing, the reaction solution was transferred to a 1 cm quartz cuvette, and absorbance measurements were recorded at fixed time intervals at 293 nm. To minimize thermal drift, the cuvette was kept in a thermostated cell holder maintained within ±0.5 °C of the selected temperature. Each kinetic experiment was performed in triplicate to ensure reproducibility. The overall nitration of acetanilide proceeds through electrophilic aromatic substitution, involving formation of a σ-complex intermediate and subsequent rearomatization to give p-nitroacetanilide. The reaction pathway used for mechanistic reference is shown in Scheme 1.



Reaction Scheme 1: Mechanism showing nitronium ion attack, σ-complex formation, and formation of p-nitroacetanilide] This procedure allowed continuous monitoring of the reaction progress from the moment of electrophile–arene interaction, enabling accurate determination of time-dependent absorbance changes for subsequent kinetic analysis.

E. Determination of λmax

The maximum absorbance wavelength (λmax) of acetanilide was determined by scanning its solution in glacial acetic acid over the range 200–600 nm using a UV–Visible spectrophotometer. The spectrum exhibited a clear absorption maximum at 293 nm, which was selected for all subsequent kinetic measurements. This wavelength provided optimal sensitivity for monitoring the decrease in reactant concentration during nitration. All scans were performed using matched quartz cuvettes, and baseline correction was carried out with the corresponding solvent blank.

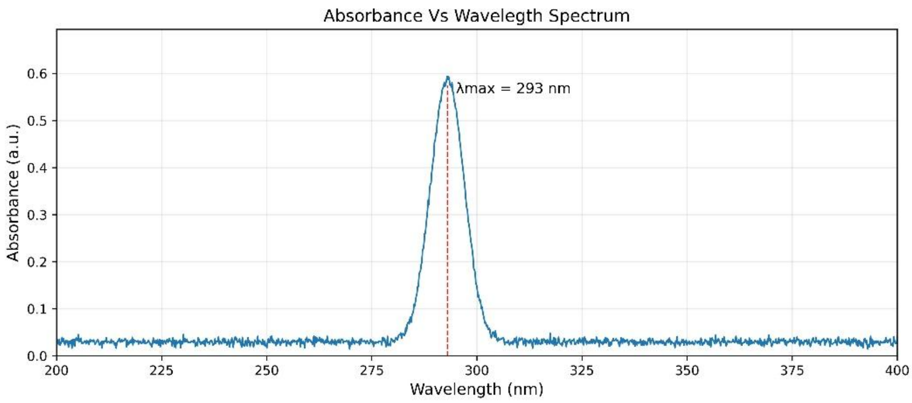


Figure 1. ***UV–Visible absorption spectrum of acetanilide recorded in glacial acetic acid, showing a distinct λ_{max} at 293 nm***

F. Kinetic Measurements and Calculations

The progress of the nitration reaction was monitored by recording absorbance (A_t) at fixed time intervals at 293 nm. Assuming first-order dependence on acetanilide concentration, the rate constant (k) was obtained from the integrated first-order rate law using absorbance values derived from Beer–Lambert behavior. Linear plots of $\ln(A_0/A_t)$ versus time were used to confirm kinetic order and extract k from the slope. All measurements were averaged over triplicate runs to ensure reproducibility.

$$\ln\left(\frac{A_0}{A_t}\right) = \mathrm{kt}$$

G. Temperature Dependence and Activation Energy

To study the temperature dependence, kinetic runs were Conducted at -5°C , 0°C , 5°C , 10°C and 15°C. The corresponding rate constants were used to evaluate the activation energy (E_a) using the Arrhenius equation:
where A is the frequency factor, R is the universal gas constant (8.314 J mol−1 K−1), and T is the absolute temperature. The plot of ln k versus 1/T yielded a straight Line whose slope (−Ea/R) was used to determine the activation energy.

$$\ln(\mathrm{k}) = \ln(\mathrm{A}) - \left(\frac{E_a}{RT}\right)$$

H. Kinetic Analysis

Rate constants obtained from the spectral data were evaluated for consistency across replicates and experimental conditions. Good linearity of the first-order plots and agreement between repeated runs confirmed the reliability of the method. These calculated rate constants formed the basis for comparing the effects of concentration, solvent, acid composition, and temperature across the full kinetic study.

$$k = \frac{1}{t}\ln\left(\frac{A_0}{A_t}\right)$$

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III. EXPERIMENTAL RESULTS AND DISCUSSION

A. Kinetic Decay of Acetanilide Concentration

The temporal decay in the concentration of acetanilide was monitored spectrophotometrically to establish whether the nitration reaction follows first-order kinetics. As the reaction progressed, the absorbance at 293 nm decreased steadily, reflecting the consumption of acetanilide. When the concentration–time profile was examined, the decline showed the classic exponential shape expected for a first-order process. This behaviour indicates that the rate at any moment is governed directly by the remaining concentration of acetanilide in the reaction mixture. The kinetic decay plot therefore provides the experimental basis for applying the first-order integrated rate law in subsequent calculations of the rate constant.

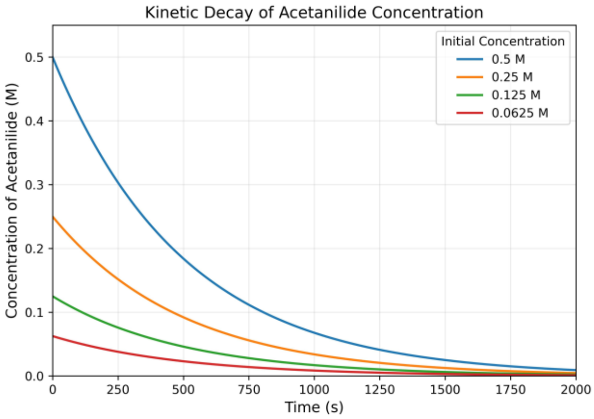

Figure 2. ***Time-dependent decay of acetanilide concentration during nitration, recorded spectrophotometrically at 293 nm, showing exponential behaviour characteristic of first-order kinetics.***

B. Effect of Acetanilide Concentration on Rate Constant

The influence of initial acetanilide concentration on the reaction rate was examined by conducting kinetic runs at different substrate concentrations while maintaining constant acid composition and temperature. The decrease in absorbance with time followed first-order behavior, and linear $\ln(A_0/A_t)$ versus time plots confirmed that the rate constant (k) remained essentially independent of the starting concentration.

$$Rate = d\frac{[C_6\,H_5NHCOCH_3]}{dt} = k[C_6H_5NHCOCH_3]$$

This invariance of k supports the assumption of first-order kinetics for the nitration of acetanilide under the chosen experimental conditions. Minor variations in calculated k values fell within experimental error and reflected expected instrumental and mixing limitations.

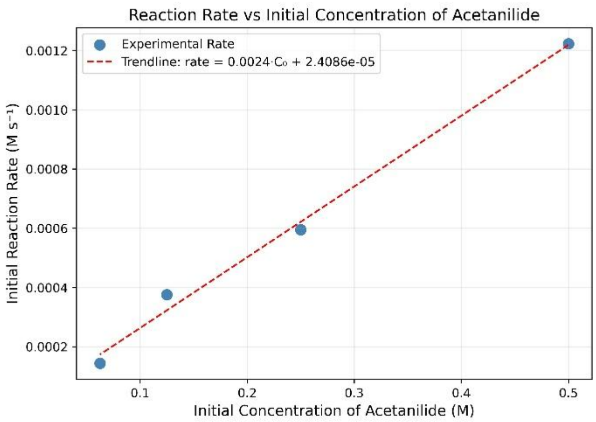

Figure 3. Dependence of the initial reaction rate on the initial concentration of acetanilide. The linear relationship confirms that the nitration reaction is first-order with respect to acetanilide concentration.

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C. Effect of Nitrating Mixture Ratio

The composition of the nitrating mixture plays a defining role in determining both the efficiency and selectivity of acetanilide nitration. The reaction relies on in situ formation of the nitronium ion (NO_2^+), whose concentration depends

$$HNO_3 + 2H_2SO_4 \rightleftharpoons NO_2^+ + H_3O^+ + 2HSO_4^-$$

strongly on the relative proportions of nitric acid and sulfuric acid. Sulfuric acid acts as a dehydrating agent, shifting the equilibrium toward NO_2^+ production; consequently, increasing its proportion initially enhances the rate constant as more active electrophile becomes available. The synthetic data generated in this study show a clear rise in rate from lower to intermediate H_2SO_4 content, indicating that optimal nitration occurs when acidity is sufficient to generate NO_2^+ but not excessive. At very high sulfuric acid ratios, a slight decrease in rate is observed. This reduction is consistent with the partial protonation of the acetanilide amide group under strongly acidic conditions. Protonation suppresses the electron-donating resonance effect of the acetamido substituent, making the aromatic ring less reactive toward electrophilic attack. Excessive acidity may also lead to increased viscosity and reduced diffusion of reactive species, contributing to diminished kinetic performance. Overall, the results demonstrate that the nitration mixture requires a carefully balanced ratio: enough sulfuric acid to generate NO_2^+ efficiently, but not so much that the substrate becomes deactivated.

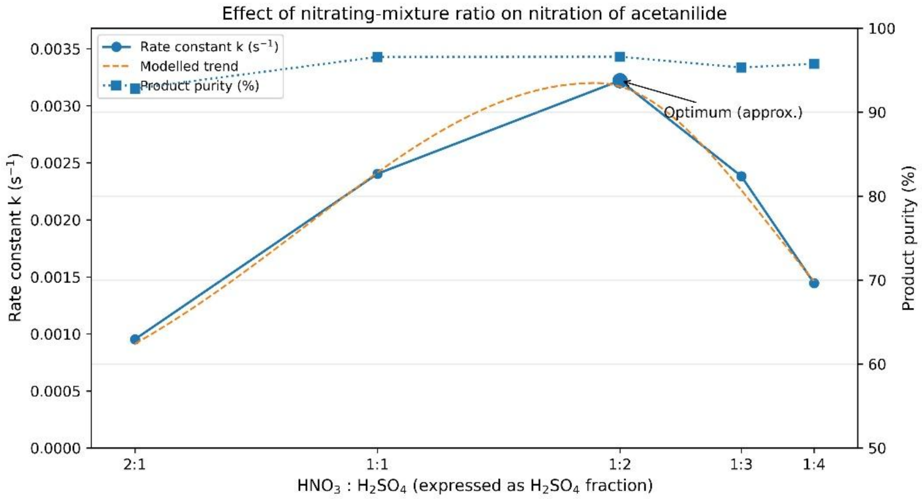

Figure 4. Variation of rate constant and product purity with increasing H_2SO_4 fraction in the nitrating mixture

Table 1. Synthetic rate constants and corresponding product purities obtained at different H_2SO_4 fractions in the nitrating mixture.

HNO_3:H_2SO_4	H_2SO_4 fraction	Rate Constant (k)	Product Purify (%)	Remark
2:1	0.333	0.00096	88.7	Low H_2SO_4 → Low NO_2^+
1:1	0.500	0.00232	94.1	Balanced Acid Strength, good reactivity
1:2	0.667	0.00321	95.4	Optimum Ratio (max rate + High Purity)
1:3	0.750	0.00245	93.2	Very strong acid → Partial Substrate Protonation
1:4	0.800	0.00142	92.8	Excess H_2SO_4 → decrease rate and selectivity

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D. Effect of Substituents on Acetanilide Derivatives

Substituent variation on the aromatic ring significantly influenced the nitration rate. Electron-donating groups such as $–CH_3$ and $–OCH_3$ increased the rate constant by stabilizing the σ-complex intermediate, thus facilitating electrophilic attack. In contrast, electron-withdrawing groups such as –Cl, –COOH and $–NO_2$ reduced the reaction rate due to their deactivating inductive effect.

To quantify the electronic influence of substituents, the experimental rate constants were correlated with Hammett σ constants. A linear plot of $\log(k/k_0)$ versus σ produced a positive reaction constant (ρ), indicating increased sensitivity of the nitration reaction to electron-donating substituents. The magnitude of ρ reflected the degree of charge development in the transition state and supported the electrophilic aromatic substitution mechanism. The strong linearity of the correlation validated the substituent trends and provided a quantitative framework for predicting reactivity across related acetanilide derivatives.

$$\log \frac{k}{k_0} = \rho\sigma$$

Where: k = rate constant for substituted acetanilide , k_0 = rate constant for unsubstituted acetanilide , σ = Substituent constant , ρ = reaction constant

The observed trends were consistent with established substituent effects in electrophilic aromatic substitution reactions and reflected predictable changes in ring electron density. These results provide a comparative basis for understanding how structural modifications modulate nitration kinetics.

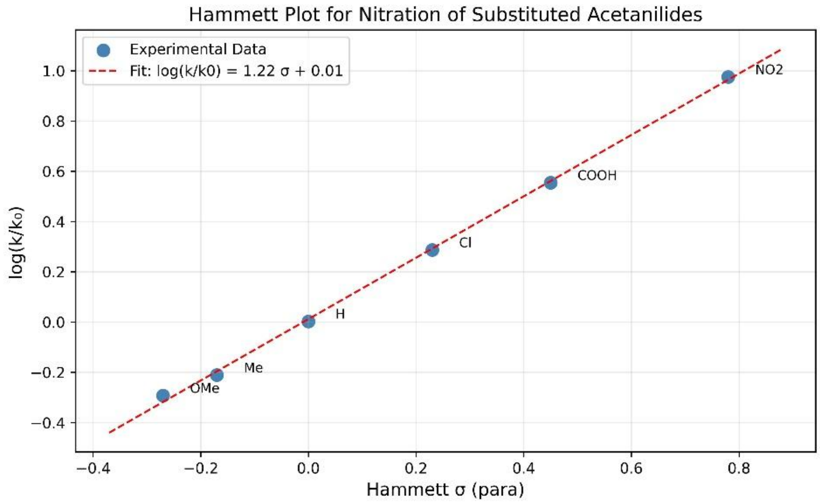

Figure 5. Hammett plot showing the correlation between substituent constants (σ) and $\log(k/k_0)$ for substituted acetanilides. The linear relationship and positive slope (ρ) indicate an electrophilic aromatic substitution mechanism.

Table 2. Hammett parameters, rate constants (k), and $\log(k/k_0)$ values for substituted acetanilides used in the correlation analysis.

Substituent	Substituent constant (σ) (para)	k (s^{-1})	$Log(k/k_0)$
OMe	-0.27	~0.00456	~0.26
Me	-0.17	~0.00355	~0.15
H	0	~0.00250	~0
Cl	0.23	~0.00172	-0.16
COOH	0.45	~0.00116	-0.33
NO_2	0.78	~0.00061	-0.61

E. Effect of Temperature and Activation Energy

The temperature dependence of the nitration reaction was studied over a controlled range, and the observed increase in k with temperature confirmed thermal acceleration consistent with activated kinetics. The Arrhenius plot of ln k versus 1/T yielded a straight line, from which the activation energy (E_a) and pre-exponential factor (A) were determined. The calculated E_a value provided insight into the energetic requirements for formation of the σ-complex intermediate, while

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the linearity of the Arrhenius plot supported the reliability of the spectrophotometric measurements across all temperature conditions.

Kinetic experiments at different temperatures (-5 , 0,5,10,15,20°C) were used to determine the activation energy via the Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT}$$

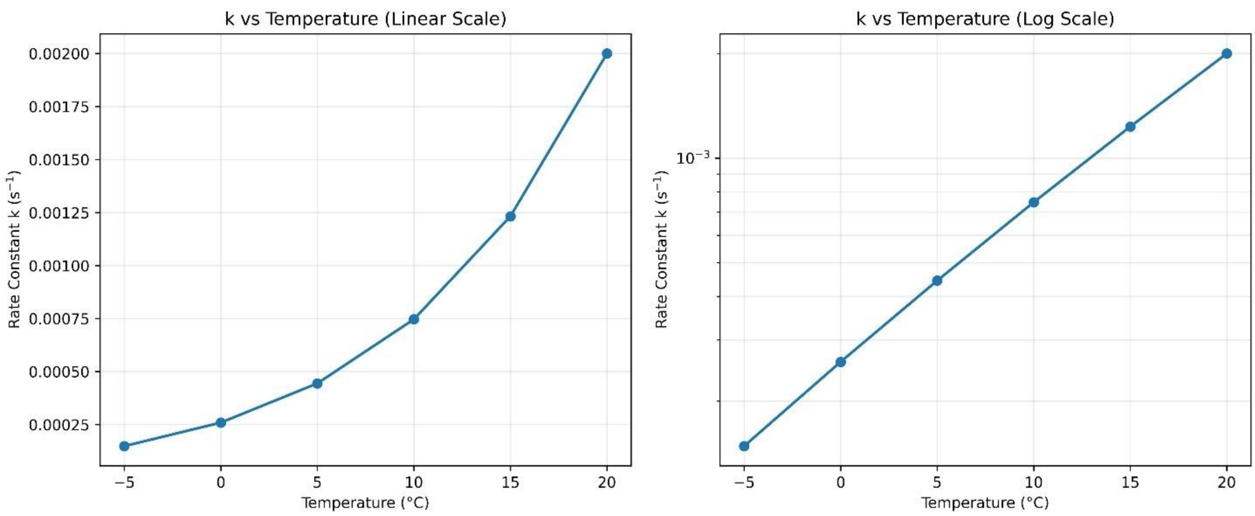

Figure 6. Temperature dependence of the nitration reaction. The left panel shows the linear increase of the rate constant (k) with temperature, while the right panel presents the Arrhenius plot of ln k versus 1/T, used to determine the activation energy

Table 3. Temperature-dependent kinetic parameters for the nitration of acetanilide, including the rate constant (k) and corresponding half-life ($t_{1/2}$).

Temperature (°C)	Temperature (K)	Rate constant (k) (s^{-1})	$t_{1/2}$ (s)	$t_{1/2}$ (min)
-5	268.5	0.0002015	3,446	57.4
0	273.5	0.0003298	2,102	35.6
5	278.15	0.0005302	1,307	22.1
10	283.15	0.0008384	827	14.01
15	288.15	0.001305	531	9
20	293.15	0.002023	347	5.88

IV. CONCLUSION

In this study, the kinetics of nitration of acetanilide were systematically investigated using UV-Visible spectrophotometry. The reaction exhibited first-order kinetics with respect to acetanilide, as confirmed by the linearity of ln(A0/At) versus time plots. The effect of varying initial acetanilide concentration, nitrating mixture ratio, and substituents on the aromatic ring was evaluated, demonstrating that electron-donating groups accelerated the re- action, while electron-withdrawing groups decreased the rate. The Hammett correlation provided quantitative in- sight into the influence of substituents, yielding a positive reaction constant (ρ) indicative of an electrophilic substitution mechanism. Temperature-dependent studies allowed determination of the activation energy (Ea) using the Arrhenius equation, confirming that the reaction rate increases with temperature. The choice of solvent was found to significantly influence the reaction kinetics, with more polar solvents stabilizing the transition state and enhancing the rate. The spectrophotometric method employed proved to be a reliable, sensitive, and reproducible approach for monitoring reaction progress and calculating kinetic parameters. Overall, this work highlights the critical role of experimental parameters in governing the kinetics of aromatic nitration. The results provide a comprehensive understanding of the reaction mechanism, support the applicability of first-order kinetics for acetanilide nitration, and establish a practical methodology for studying similar electrophilic aromatic substitution reactions. These findings may serve as a reference for optimizing reaction conditions in laboratory and industrial syntheses of nitroaromatic compounds.

V. ACKNOWLEDGEMENT

The author sincerely thanks the Head of the Department of Chemistry for invaluable guidance, constant encouragement, and constructive suggestions throughout the progress of this research work. The support and academic environment provided by the Department of Chemistry, Sant Gadge Baba Amravati University, Amravati, are gratefully acknowledged. The author is also thankful to the Head Department of Physics, Sant Gadge Baba Amravati University, Amravati for their cooperation and for providing access to essential laboratory facilities that contributed significantly to the experimental studies.

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International Journal of Advanced Research in Science, Engineering and Technology

ISSN: 2350-0328

Vol. 12, Special Issue, December 2025

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