JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد السابع والاربعون
٢٠٢٦م /١٤٤٨هـ
العدد السابع والاربعون
(573) (551)

استراتيجية اقتران الآزو المائي لتحديد ٣-أمينوفينول: التركيز على استقرار التفاعل والتطبيق البيئي العملي

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المستخلص:

في هذا البحث، تم وصف تصميم طريقة طيفية ضوئية (Spectrophotometric) لتحليل مركب ٣-أمينوفينول (AP-٣) في المحاليل المائية، حيث استُخدم تفاعل الاقتران الآزوي لـ ٣-أمينوفينول في وسط قلوي لتحليل المركب. وقد تكوَّنت صبغة آزوية برتقالية مستقرة ذات أعلى طول موجي للامتصاص عند ٤٥٨ نانومتر. أظهرت نتائج التحليل التزامًا دقيقًا بقانون بير-لامبرت ضمن مدى تراكيز يتراوح بين ١-١٢ مايكروغرام/مل، كما أظهرت الطريقة كفاءة إحصائية عالية بمعامل تحديد بلغ $R^2 = 0.9933$، مما يدل على دقة العلاقة الخطية وجودة منحنى المعايرة. تضمنت التجربة تحسينًا واسعًا لظروف التفاعل، بما في ذلك طبيعة وتركيز القاعدة، وثبات التفاعل، وتسلسل إضافة الكواشف، كما أظهرت التفاعلات الستوكيومترية أن النسبة المولية للمعقد كانت ١:١. بلغ حد الكشف ٠.٠١٤١ مايكروغرام/مل، وحد التقدير الكمي ٠.٠٤٧٢ مايكروغرام/مل، مع حساسية عالية للكشف عن الملوثات ذات التراكيز المنخفضة. وقد تم تطبيق المنهجية المقترحة بنجاح لتقدير المركب في عينات بيئية حقيقية، مثل مياه نهر ديالى، ونهر خريسان، وبحيرة حمرين، مع نسب معدل استرجاع ١٠٢.٩١ وانحراف معياري نسبي منخفض (RSD < 1.5%). وتُعد هذه الطريقة منخفضة التكلفة وفعالة للمختبرات التحليلية، لكونها سهلة التطبيق، ولا تتطلب طرائق استخلاص معقدة أو أجهزة باهظة الثمن، كما يمكن اعتمادها في الإدارة البيئية اليومية لمراقبة جودة المياه.

الكلمات المفتاحية: ٣-أمينوفينول، التقدير الطيفي اللوني، تفاعل الاقتران الآزوي، الديازتة، تحليل المياه البيئية

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Aqueous Azo-Coupling Strategy for the Determination of 3-Aminophenol: Emphasis on Reaction Stability and Practical Environmental Application

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Abstract:

In this paper, the design of a spectrophotometric process for analyzing the 3-aminophenol (3-AP) in aqueous solutions is described, where the azo-coupling reaction of 3-AP, and an alkaline medium is used to analyze 3-AP and the alkaline medium, respectively. It forms a stable orange azo dye with a maximum absorption wavelength of 458 nm. The results of the analysis showed that the strict adherence of the Beer-Lambert law was observed in the range of concentrations ranging between 1-12 µg/mL, and it showed high statistical efficiency with a coefficient of determination of R2 = 0.9933, which indicates the accuracy of the linear relationship and the quality of the calibration curve. The experiment entailed a massive optimization of the reaction conditions, including the nature and concentration of the base, stability, and sequence of addition of the reagents, and stoichiometric reactions had a 1:1 molar ratio of the complex. The limit of detection was 0.0141 µg/mL, and the limit of quantification was 0.0472 µg/mL, with high sensitivity to pollutants in low concentrations. The proposed methodology has been successfully applied to determine the compound in real environmental samples, such as water from the Diyala River, Khresan River, and Hamrin Lake, with a average recovery was found to be 102.91% and a small relative standard deviation (RSD). It is a low-cost and efficient method for analytical labs because it is easy, does not involve complex ways to extract or expensive devices, and can be employed in daily environmental management to check the water quality.

Keywords: 3-Aminophenol, Spectrophotometric determination, Azo-coupling reaction, Diazotization, Environmental water analysis.

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1. Introduction

Organic water pollutants, particularly in phenolic and amino derivatives, are an important challenge to modern analytical chemistry due to their chemical stability and cumulative toxicity (Andrés et al., 2025; Edebali et al., 2024). Among them, the most significant is the aromatic bifunctional molecule 3-aminophenol (3-AP), which has the amino (−NH2) and the hydroxyl (−OH) groups directly attached to the benzene ring (Sahar et al., 2024). However, it has been widely used in chemistry, thus leading to its unlimited leaching into water bodies and industrial effluents (Roustaei et al., 2024; Tabaraki & Heidarizadi, 2019). Metal, as a dangerous pollutant, affects biological processes (Mohammed, 2025). High-precision, sensitive, and rapid monitoring protocols must be developed for exposure to π-conjugation, given that chronic exposure could pose health risks, including methemoglobinemia, oxidative stress, and possible bladder carcinogenesis (Oueslati et al., 2025). Recently, several contemporary separation and analysis techniques, such as gas chromatography mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and capillary electrophoresis, have found their way into the scientific community, although their use has been criticized due to their lack of economic sustainability and the sheer extraction costs and high volumes of organic solvents needed (Catarro et al., 2025; Soliman et al., 2004). In order to surpass this shortcoming, an interest in optimization of spectrophotometric (UV Vis) techniques as a more environmentally friendly and more accessible alternative has returned, using simplicity, speed, and ease-of-use (Mustafa et al., 2024; Poonguzhali et al., 2023). Spectrophotometric estimation of 3-AP is mainly based on various derivatization reactions (Han & Gysi, 2024), however, in these cases the azo-coupling reaction is the most popular as it enables the formation of stable chromophores with very high molar absorptivity, making it possible to perform quantitative analysis even at trace level (Schram et al., 2024). The azo-coupling reaction is based on a two-step reaction mechanism, involving the conversion of the primary amine to a highly reactive diazonium on an acidic solution (Fadhel & Hamdani, 2024), which then undergoes an electrophilic attack on an activated aromatic ring to

give a new structure with a higher level of 3p-conjugation (Sengupta & Das, 2022). This response is very medium-dependent; the strength of the acid can stabilize the diazzonium ion (Yang et al., 2024), and the base can affect the coupling step by increasing the electron density of the aromatic ring (Abd El-wahaab et al., 2020), despite the fact that some of the studies have addressed these deficiencies; they are limited by the complex nature of the environmental matrices in terms of linear range (or sensitivity) (Dehghani et al., 2024; Yadav et al., 2019). Therefore, there is a need to develop a methodology that can overcome these limitations, particularly the limited linear range and sensitivity associated with complex environmental matrices, through comprehensive optimization of the reaction conditions and stabilization of the parameters affecting the optical signal intensity (Ji et al., 2025; Wang et al., 2022). In this study, we outline an economic, precise, fast and sensitive spectrophotometric determination of 3-aminophenol achieved after optimization of an azo-coupling reaction with a considerable amount of experiment design and an effective although simple analytical protocol that is more stable in color and is best applicable to regular monitoring in the environmental water**.**

2. Experimental

2.1. Instrumentation

Spectrophotometric Measurements: All absorbance measurements and spectral scans were performed on Shimadzu UV-1800 double beam spectrophotometer (Japan). This was done under conditions appropriate for 1 cm-1 path length quartz cells (Hewlett-Packard, Agilent, Santa Clara, CA) using scans in the ultraviolet and visible range (200–800 nm).

Solid reagents were weighed using a Precisa analytical electronic balance (Switzerland) with a sensitivity of ±0.0001 g.

pH measurements: The pH values of the various solutions were monitored and adjusted using a pH-meter (WTW, Germany).

Thermal Control: Diazotization and coupling stages needed specific temperature so a calibrated Water Bath was used to provide it (VELP SCIENTFICA MADE IN URPOE).

2.2. Regents and materials

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All chemicals used in this study were of analytical grade and were obtained from internationally accepted suppliers e.g., (Merck, sigma -Aldrich).

For 3aminophenol (3-AP, Merck, Germany): A standard stock solution (100 µg/mL) was prepared by dissolving 0.01 g in distilled water.

Reagent: 3-Nitroaniline (Sigma-Aldrich, USA): 0.001 M in distilled water (primary source of diazonium ion)

Formation of Diazonium Salts: Sodium Nitrite (NaNO2): A freshly prepared solution (1% (w/v)) in water was prepared daily before the diazotization step to ensure its accuracy.

Reagents and Media: Hydrochloric acid solution (1 M and 2 M), Sodium hydroxide solution (1 M and 2 M) were used to establish optimum acid-base environment for the reaction.

2.3. General analytical procedure

The spectrophotometric determination of 3-aminophenol (3-AP) was carried out using the following optimized procedure. A suitable volume of 3-nitroaniline reagent was transferred into a 25 mL volumetric flask, followed by the addition of HCl (1 M) and $NaNO_2$ (1%) at volumes of 1.0 and 0.8 mL, respectively. The mixture was then cooled for 5 min in an ice bath at 0–5 °C to stabilize the diazonium salt. Subsequently, a suitable volume of 3-aminophenol (3-AP), the analyte, within the linear range of 1–12 µg/mL was added. Then, 1.5 mL of KOH (1 M) was added to provide the alkaline medium required for the azo-coupling reaction and formation of the colored azo dye. The volume was then made up to the mark with distilled water. The absorbance was measured at λmax = 458 nm against a reagent blank prepared in the same manner but without 3-aminophenol (3-AP), after an appropriate stabilization period.

3. Results and discussion

3.1. Absorption spectrum

The spectrophotometric method develops on the basis of coupling reaction of diazotized 3-nitroaniline with 3-aminophenol in alkaline medium leading to the formation of colored azo dye. The maximum absorption wavelength λ max was determined by making a spectral scan in the visible range for both the obtained product and the corresponding reagent blank. Azo dye shows a

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Online-ISSN 2791-3279
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sharp and strong absorption peak at 458 nm, Figure 1. The reagent blank, on the other hand, showed minimal absorbance at this wavelength. Such a large bathochromic shift indicates the development of an extended conjugated chromophoric system and provides the high sensitivity and selectivity needed for direct quantitative determination of 3-AP, without significant spectroscopic interference of the reagents.

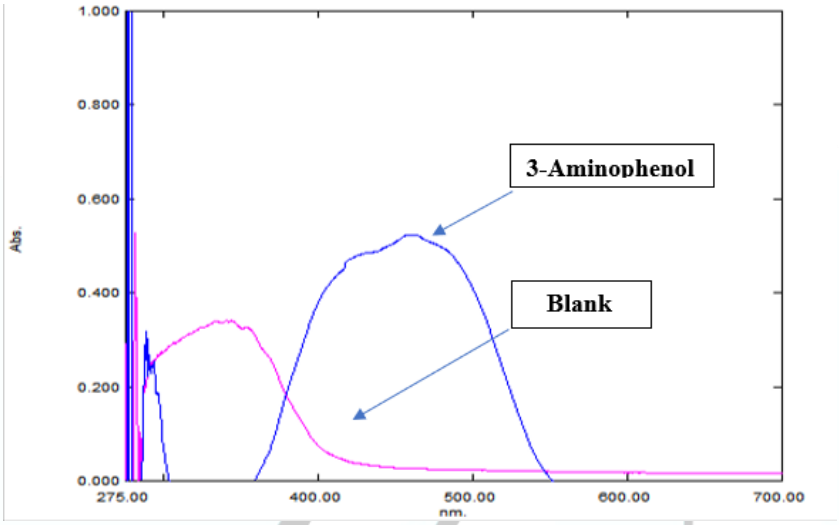

Figure 1: Absorption spectra of the formed azo dye versus reagent blank, with λ max appearing at 458 nm(٥٠ °C)

٣.2. Proposed reaction mechanism

A two-step mechanism is proposed according to the chemical properties of the reactants and the spectral data gained, Scheme 1:

Step of Diazotization — The first aromatic amine (3-nitroaniline) is reacted with sodium nitrite in an acidic medium (HCl) at a low temperature (0-5 °C) to generate stable and highly reactive 3-nitrobenzenediazonium chloride salt.

Step of Azo-Coupling: The diazonium cation attacks electrophilically the ring of the aromatic compound 3-aminophenol (in an alkaline medium (KOH/NaOH). These two groups (-OH and -NH_2) have shown strong activating character (ortho/para) causing the coupling to occur at room temperature (Scheme 1) leading to stable Dark Orange azo dye. The outstanding color and molar absorptivity relate to the strong extensive π-conjugation across the azo bond (N=N).

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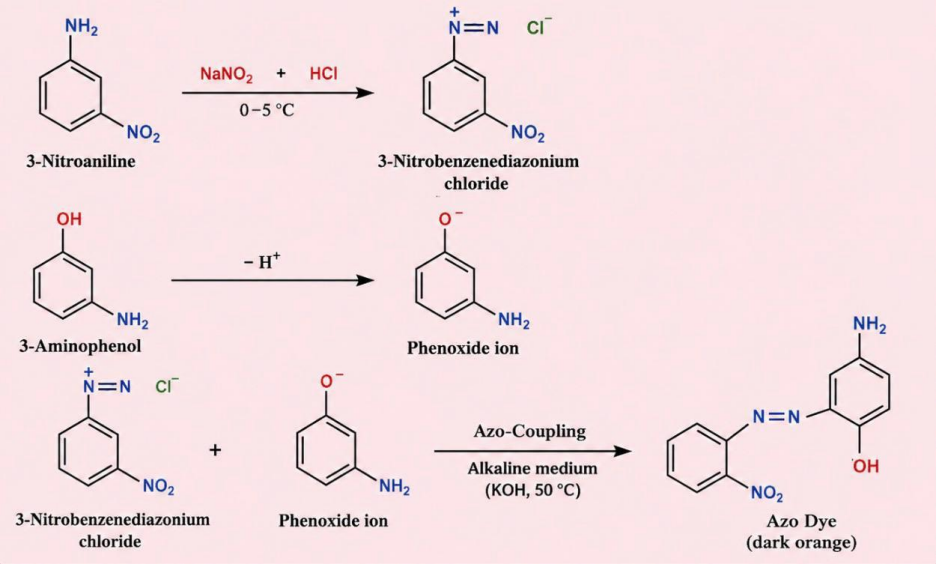

Scheme 1: Proposed reaction mechanism for the formation of the azo dye between diazotized 3-nitroaniline and 3-aminophenol

3.3. Optimization of reaction conditions

3.3.1. Optimization of acid and base type

As depicted in Table 1, various acids were tested, and the findings revealed that HCl produced the greatest absorption as compared to the other acids. This is because it is highly efficient at providing the optimal acidic medium for the diazotization process, producing a more stable diazonium salt. Regarding these bases, as illustrated in Table 1, various bases were tested, and KOH showed the highest absorbance among the bases tested. This is attributed to its ability to provide the right alkaline environment, which catalyzes 3-aminophenol into an azo compound, thereby increasing its coupling with azo to produce a more intense and stable dye.

Table 1: Optimization of acid and base types for the azo dye formation at 458 nm

Material Type		Absorbance at 458 nm
Acid	HCl	0.302
	H_2SO_4	0.287
	H_3PO_4	0.245
	CH_3COOH	0.292
Base	NaOH	0.317

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Print -ISSN 2306-5249
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	KOH	0.337
	$Ba(OH)_2$	0.285
	$NaHCO_3$	0.304

3.3.2. Optimization of acid and base volume

At a constant volume between 0.1–1.0 mL, the reagent has a large effect on absorbance. The most complete formation of the dye was attained with the addition of 0.6 mL of 1M HCl, resulting in the highest absorbance of 0.317 under the acid medium conditions. Extending the dilution beyond this just above this point resulted in a minor decrease in color intensity likely from excess acid disturbing the conjugation system. As shown in Table 2, The basic environment using of 0.7 mL 1M KOH gave a better chromatic response with an absorbance of 0.365. This implies that the alkaline surroundings maximize the charge redistribution in the chromophore molecule and stabilize it (Darweesh, 2017).

Table 2: Optimization of acid and base volume for the azo dye formation at 458 nm

Volume of 1M acids	**Absorbance** of HCl at λ max =458	**Absorbance** of KOH at λ max =458
0.1	0.195	0.279
0.2	0.203	0.3
0.3	0.211	0.311
0.4	0.225	0.327
0.5	0.301	0.344
0.6	0.317	0.315
0.7	0.31	0.365
0.8	0.308	0.355
0.9	0.304	0.348
1.0	0.301	0.337

3.3.3. Optimization of $NaNO_2$, urea, and the coupling reagent volumes

The results presented in Table 3 demonstrate that the absorbance intensity was affected by the volumes of the reagents used in the reaction. The

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optimum volume of sodium nitrite ($NaNO_2$) was 0.8 mL, which produced an absorbance of 0.382. This result can be attributed to the availability of a sufficient amount of nitrite ions to promote complete diazotization of the 3-nitroaniline reagent. An insufficient amount of $NaNO_2$ may result in incomplete diazotization, whereas an excess amount may adversely affect the formation and stability of the diazonium salt (Hassan & Mizher, 2018). Moreover, the addition of 0.3 mL of urea increased the absorbance to 0.420. This effect can be attributed to the ability of urea to remove excess nitrous acid (HNO_2) remaining after diazotization, thereby minimizing unwanted reactions and providing suitable conditions for the subsequent azo-coupling reaction (Hassan & Al-Rubaiawi, 2017). Regarding the coupling reagent, the highest absorbance (0.451) was obtained at a volume of 0.5 mL. This result indicates that this volume provided suitable conditions for efficient coupling between the diazonium species and 3-aminophenol, resulting in the formation of the colored azo chromophore. Therefore, 0.8 mL of $NaNO_2$, 0.3 mL of urea, and 0.5 mL of the coupling reagent were selected as the optimum volumes for subsequent experiments.

Table 3: Optimization of reagent volumes for the azo dye formation at 458 nm

Volume of 1M acids	Abs of $NaNO_2$	Abs of urea	Abs of reagent
0.1	0.281	0.407	0.332
0.2	0.298	0.412	0.387
0.3	0.311	0.42	0.404
0.4	0.32	0.415	0.427
0.5	0.336	0.41	0.451
0.6	0.341	0.406	0.448
0.7	0.354	0.4	0.436
0.8	0.382	0.398	0.431
0.9	0.374	0.39	0.422
1.0	0.366	0.382	0.42

٣.3.4. Optimization of reaction time

Figure 2 demonstrates, the absorbance intensity increases gradually with time, indicating the slow character of the coupling process between the diazonium ion and 3-aminophenol. The maximum absorbance (0.502) was reached after 50 minutes, the time during which the whole chromophoric system of the azo dye was formed. This is chemically explained by the fact

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Print -ISSN 2306-5249
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that the electrophilic aromatic coupling reaction requires sufficient time to allow the reactants to fully interact with each other and to stabilize the azo bond (N=N) in the conjugated system. As observed in the curve, the absorbance began to increase after 50 minutes, which is chemically explained by the fact that once the dye structure had reached its peak stability, gradual degradation began, or the dye's sensitivity to the environment. Thus, 50 minutes was the best reaction time to ensure maximum accuracy across all the measurements.

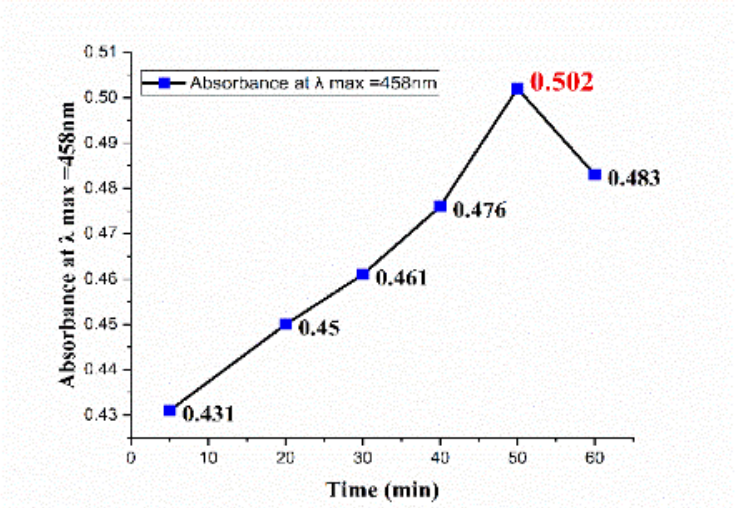

Figure 2: Effect of reaction time on absorbance of the formed azo dye at 458 nm

3.3.5. Optimization of temperature

The results in Figure 3 show a significant effect of temperature changes on the dye's absorption intensity. The color reaction was the highest at (50 °C) with an absorption of 0.554. This rise is chemically attributed to the fact that the observed temperature rise lowers the activation energy required for the electrophilic coupling reaction, which in turn accelerates the formation of the chromophore system of the dye. Nevertheless, the graph showed a decrease in readings after this temperature limit. This can be explained by the sensitivity of diazonium salts, which are easily broken down by thermal processes at high temperatures, and the likelihood that the resonant stability of the azo bond is damaged by overheating. According to these results, 50 °C

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was determined to be the optimal temperature for maximum accuracy and sensitivity in the analysis

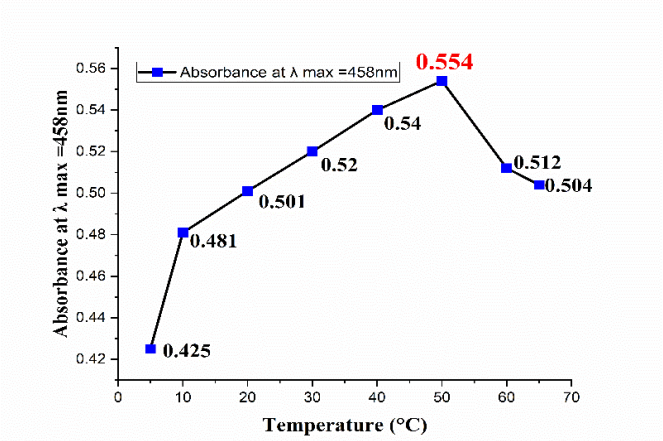

Figure 3: Effect of temperature on absorbance of the formed azo dye at 458 nm

3.3.6. Optimization of solvent type

From Table 4, it is observed that water showed highest absorbance (0.565), which indicates that the reaction takes place under most favourable conditions in aqueous environment. This is due to the highest polarity of water, which makes the ionic diazonium intermediate as well as the chromophore formed as stable as by efficient solvation and hydrogen bonding. On the other hand, organic solvents such as ethanol and acetone resulted in a less intense response due to their lower dielectric constants, which reduces the stabilization of the ionic species.

Table 4: Effect of solvent on absorbance

Solvent	Absorbance at 458 nm
Water	0.565
Ethanol	0.532
Methanol	0.442
Acetonitrile	0.468
1-Propanol	0.522
Acetone	0.537

3.3.7. Optimization of addition sequence

Different addition sequences were also studied in order to reach a higher efficiency of reaction and the results are presented in Table 5. The 0.503 of absorbance was highest obtained from sequence (A+B+C+D+E+F) respectively. This exact setup is the best because it secures you perform

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diazotization reaction entirely in acid prior to applying urea to neutralize redundant nitrite. Most sequences gave absorbance values which were substantially lower, such as 0.342 and 0.247 because changing the steps destabilizes the diazonium salt or impairing the dye formation. For all experiments, this sequence was constant in order to provide optimal sensitivity and stability.

Table 5: Effect of addition sequence

Addition	Absorbance at 458nm
A+B+C+D+E+F	0.503
A+F+E+C+D+B	0.342
A+E+B+C+D+F	0.247

Note: A: 3-nitroaniline, B: HCL, C: $NaNO_2$, D: Urea, E: 3-aminophenol, F: KOH

3.4. Stoichiometric ratio (Job's method)

The stoichiometry relationship between the 3-aminophenol and the diazotized reagent was conducted by Job's plot as shown in Figure 4. The resulting profile displayed a peak absorbance at a mole fraction around 0.563, which indicates a 1:1 molar ratio corresponding to the azo dye formation. This stoichiometry explains the formation of the chromophore, one mole of the diazonium ion is reacted with one mole of 3-aminophenol. Indeed, this allows considering such a stoichiometric interaction in the framework of the proposed mechanistic model where the diazonium cation acts as an electrophile attacking the activated aromatic ring. In addition, the sharp peak in the Job's plot testifies to the stability of the formed complex and the lack of side reactions in the optimized experimental design (Abbas, 2017).

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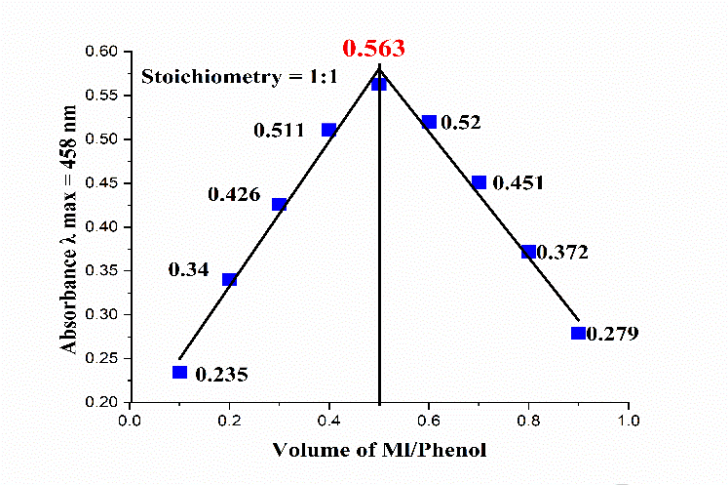

Figure 4: Determination of the stoichiometric ratio of the azo dye using the continuous variation method

3.5. Stoichiometric ratio (Mole ratio method)

The corresponding curve Figure 5 show a linear rise in absorbance followed by a rapid change in slope and an inflection point at approximately a 1:1 molar ratio. This observation shows that at the equivalence point the diazonium ion's constant concentration becomes the driving force that is saturated. The data obtained this way fit precisely with the results over the plot Job, reinforcing the conclusion as only one azo linkage is formed per molecule. This coherence confirms the suggested reaction mechanism and ensures that the system follows a predictable coupling mode (Ali & Muhammed, 2018).

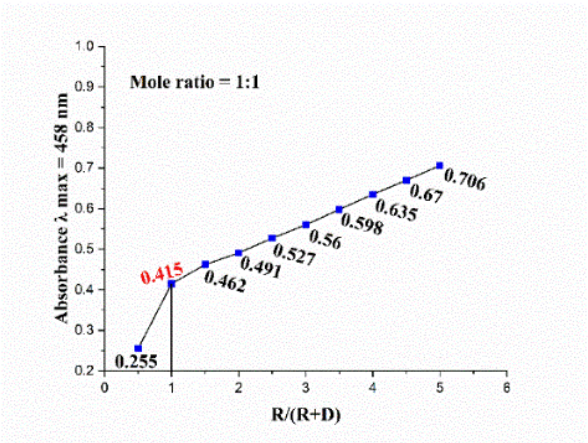

Figure 5: Determination of stoichiometry for the complex via mole ratio method

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3..6. Stability constant of the azo dye

The stoichiometry and the stability constant (K) of the synthesized azo dye are presented in Table 6. The ratio is also confirmed by the Job's method as well as mole ratio methods, confirming accordingly a 1:1 molar ratio consistent with the suggested reaction mechanism. Such stability constant mean value is 4.3×10^3 L·mol^{-1} pointing out the thermodynamic stability found for the dye onto support under the optimized of experimental conditions. Such high K value indicates predominance of an electrophilic interaction between the diazonium ion and 3-aminophenol, as several electron donating substituents (–OH and –NH_2) activating the aromatic ring. Additionally, the azo bond's facilitating an π-conjugated system allows the structure to stabilize through resonance which assists in the color depth.

Table 6: Stability constant of the formed azo dye

Parameter	Value
Stoichiometric ratio	1:1
Mean stability constant	4.3×10^3 L·mol^{-1}

3..7. Analytical performance and calibration curve

As illustrated in Figure 6. The absorbance of 3-Aminophenol concentration ranging from 1–12 µg/mL showed a linear relationship, and the results show that Beer-Lambert's law is obeyed by the method. A high sensitivity is indicated by the slope (0.0484), and a very small intercept (0.0117) suggests a low impact of the reagent blank. The correlation coefficient (R^2 = 0.9933) indicates excellent linearity between the variables and a high degree of proportionality. The formation of the azo chromophore is stable, and the coupling was efficient, which contributes to the reliable performance.

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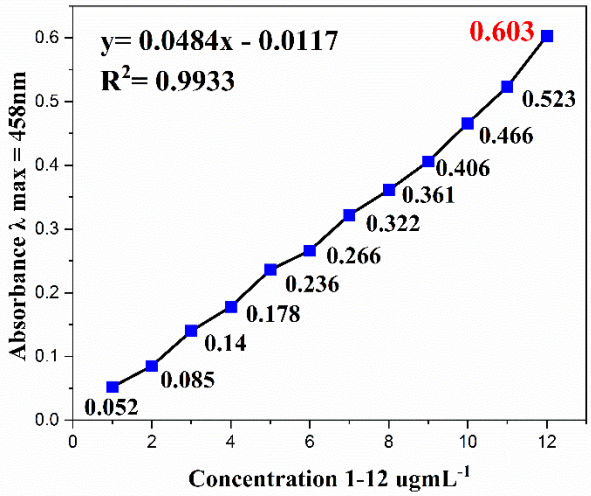

Figure 6: Calibration curve of 3-Aminophenol

3..8. Effect of interferences

Table 7 results indicate that the method used in the analysis is selective for 3-aminophenol at 458 nm, as demonstrated by experiments with a combination of aromatic compounds competing with 3-aminophenol. Compounds like aniline (0.121) and benzoic acid (0.102) showed that their absorbance values changed significantly, thus indicating that they were less efficient in forming a stable chromophore system with the reagent under the current conditions of the reaction than the analyte under investigation. In the case of 2-nitrophenol, the absorbance value was found to be (0.451), which was quite near to the absorbance value of the base analyte (0.473). This is chemically explained by their structural similarity, which allows partial coupling with the diazonium salt. The selectivity of the method was however tolerable since choice of wavelength was the best and the reaction conditions were regulated, which highly reduced the impact of such interferences. This is also confirmed by the error of less than 2% in the analytical estimation of environmental samples. These data demonstrate the method's effectiveness in separating the analyte from common interfering substances that can coexist with it under aqueous conditions.

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Table 7: The effect of interferences on the absorbance 3-aminophenol

NO.	Interference	Abs.
1	Aniline	0.121
2	Benzoic acid	0.102
3	2-nitrophenol	0.451
4	p-nitrophenol	0.231
5	Without	0.473

3..9. Analytical and statistical validation

Validity for the λ max= 458 nm with performance parameters as shown in Table 8 proves the reliability of the method. Linearity of the method was found to be excellent (r = 0.9966) for 1–12 µg/mL Low LOD (0.0141 µg mL^{-1}) and LOQ (0.0472 µg mL^{-1}) indicated high sensitivity. The resulting molar absorptivity showed 5287 $L \cdot mol^{-1} \cdot cm^{-1}$, which confirms a highly effective dye formation. In addition, the average recovery was found to be 102.91% and the low value of RSD (0. 25025%) underlines a high level of precision and accuracy. All measurements were performed in triplicate (n=3). The statistical outcomes confirm that the procedure is reliable and appropriate for the trace analysis of 3-aminophenol in the environmental samples .

Table 8: Analytical and statistical performance of the proposed method

Parameter	Value
λmax	458 nm
Linear range	1–12 µg mL^{-1}
Regression equation	y = 0.0484x + 0.0117
Correlation coefficient (R^2)	0.9933
Limit of Detection (LOD)	0.0141 µg mL^{-1}
Limit of Quantification (LOQ)	0.0472 µg mL^{-1}
Molar absorptivity	5287 $L \cdot mol^{-1} \cdot cm^{-1}$
Sandell's sensitivity	0.02064 µg cm^2
Average Recovery (%)	102.91

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Relative Error (%)	2.91
RSD (%)	0. 25025
Confidence limit. at 3 µg mL^{-1}	3.1854 ± 0.00312
Confidence limit. at 6 µg mL^{-1}	6.1606 ± 0.05194
Confidence limit. at 9 µg mL^{-1}	9.1337 ± 0.01344
Confidence limit. at 12 µg mL^{-1}	12.160 ± 0.11738

3.10٠. Application to environmental water samples

Table 9 shows that the developed analytical method was effective when used on different actual environmental samples, such as Diyala River, Khresan River, and Lake Hamrin. The percent recovery was 100.13 to 101.59, confirming that the method is very accurate and the percentage of the reagent response is not significantly influenced by a large matrix effect. The analysis of performance across environments reveals slight variations, demonstrating that the approach can be applied to different water matrices. The Diyala River had the greatest relative error (1.416%) as the flowing water is subject to this, and it may be turbid. Khresan River was found to be more stable, with a recovery percentage of 100.79, and Lake Hamrin was found to be the most accurate, with a perfect recovery percentage of 100.13, due to the physical and chemical stability of the stagnant water environment. This performance stability demonstrates the approach's ability to withstand the complexity of the aqueous matrix. The relative error rates of up to 1.5, which did not exceed 1.5, confirm that there were no systematic errors in the results and, therefore, the given spectroscopic method is a useful tool for the quantitative estimation of 3-aminophenol in the daily environmental monitoring.

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Table 9: Application of the proposed method to environmental water samples

Water source	Amount added (µg ml^{-})	Amount found (µg ml^{-})	Recovery %	Average Recovery %	E_{rel}%	Average E_{rel} %	RSD %
Diyala river	20	20.2480	101.2400	101.4160	1.2400	1.4160	0.0038
	10	10.1529	101.5920		1.5920		0.0042
Khresan river	20	20.1084	100.5420	100.7956	0.5420	0.7956	0.0009
	10	10.1049	101.0492		1.0492		0.0012
Lake Hamrin	20	20.0280	100.1401	100.1370	0.1401	0.13705	0.0017
	10	10.0134	100.1340		0.1340		0.0008

3.11. Comparative evaluation of the proposed method with reported analytical procedures

The analytical performance of the devised method is compared with other methods outlined for determining aminophenol in water samples, as shown in Table 10. The recorded data show that the sensitivity of the proposed approach was 0.0141 g/mL, which is lower than reported by literature method (22) and (23). The developed chromophore system could be efficient in detecting low concentrations within the specified range. Another outcome was high accuracy with an RSD% of less than 1.5%, which was competitive compared to the other methods, which showed RSD% between 2.4 - 3.7%. The results showed that the recovery% was in the range 100.13-101.59, indicating a reasonable level of accuracy. These values are consistent with

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those of the reference studies and fall within the acceptable analysis range. Based on these results, the proposed solution strikes a golden mean between sensitivity and accuracy in analytical performance, while also simplifying water sample analysis and eliminating time-consuming extraction procedures.

Table 10: Comparison of the proposed method with reported analytical methods

Analytical parameters	Present method	Literature method (Imran, A. M., et al. 2024)	Literature method (Asghari et al., 2022)
Technique	UV-Vis (Azo)	CPE-UV-Vis	USAEME-HPLC
Beer's law range (µg/ml)	1.0 - 12	5.0 - 14	0.085 - 157
LOD (µg/ml)	0.0141	0.0423	0.028
RSD (%)	< 1.5%	2.4 - 3.31	3.7
Recovery (%)	100.13 - 101.59	87.2 - 95.43	98.6 – 101.7
Application	Water samples	Water samples	Water samples

4. Conclusion

This work developed an accepted spectrophotometric system for the determination of trace amounts of 3-aminophenol in environmental water systems. The procedure is based on a defined streamlined azo-coupling reaction with 3-nitroaniline, which forms a stable chromophore for quantification. The performance regarding analytical precision is high, with a strong linear correlation, as indicated by a coefficient of determination (R^2 = 0.9933), granting high precision and statistical reliability. Experiments in the field on Diyala River, Khresan River, and Hamrin Lake showed a steady recovery and low interaction between matrices under realistic conditions. This method eliminates the use of hazardous organic solvents, complex extraction procedures, or costly chromatographic instruments, making it a sustainable and cost-efficient alternative for environmental laboratories. The permanence of the developed azo dye and the method's ability to withstand typical interferences validate its applicability for routine monitoring and

quality control of natural water resources against industrial phenolic contaminants .

REFERENCES

1. Abd El-wahaab, B., Elgendy, K., & El-didamony, A. (2020). Synthesis and characterization of new azo-dye reagent and using to spectrophotometric determination of samarium (III) in some industrial and blood samples. Chemical Papers, 74(5), 1439-1448 .

2. Abbas, R. F. (2017). Spectrophotometric determination stability constant by classical and modified Varagas equations for procaine penicillin G using diazotization reaction depending on stoichiometric curves. International Journal of ChemTech Research, 10(3), 485–496.

3. Ali, H., & Muhammed, S. (2018). Use of acidic hydrolysis and diazo coupling reaction for spectrophotometric determination of furosemide in urine and pharmaceutical formulation. Ibn Al-Haitham Journal for Pure and Applied Sciences, 2017(IHSCICONF), 221–234. https://doi.org/10.30526/2017.IHSCICONF.1795.

4. Andrés, E.-R., Ignacio, M.-B. K., & Maribel, M.-T. (2025). Aromatic amines in the environment: ecotoxicological effects in vertebrates and invertebrates: a review. Chemistry and Ecology, 41(8), 1115-1149 .

5. Asghari, A., Fazl-Karimi, H., Barfi, B., Rajabi, M., & Daneshfar, A. (2022). Application of ultrasound-assisted emulsification microextraction for simultaneous determination of aminophenol isomers in human urine, hair dye, and water samples using high-performance liquid chromatography. Human & experimental toxicology, 33(8), 863-872 .

6. Catarro, G., Pelixo, R., Feijó, M., Rosado, T., Socorro, S., Araújo, A. R., & Gallardo, E. (2025). Analytical Approaches Using GC-MS for the Detection of Pollutants in Wastewater Towards Environmental and Human Health Benefits: A Comprehensive Review. Chemosensors, 13(7), 253 .

7. Dehghani, F., Yousefinejad, S., Mobaraki, N., Nekoeinia, M., & Hemmateenejad, B. (2024). Rapid determination of Hippuric acid as an exposure biomarker of toluene using a colorimetric assay and comparison with high-performance liquid chromatography. Scientific Reports, 14(1), 9935.

8. Darweesh, S.A. (2017) 'Spectrophotometric determination of cefixime following simple diazotization and coupling with α-naphthol', Iraqi Journal of Pharmaceutical Sciences, 26(2), pp. 1–6. doi: 10.31351/vol26iss2pp1-6 .
9. Edebali, O., Krupcikova, S., Goellner, A., Vrana, B., Muz, M., & Melymuk, L. (2024). Tracking aromatic amines from sources to surface waters. Environmental Science & Technology Letters, 11(5), 397-409 .
10.Fadhel, A. M., & Hamdani, A. (2024). Al Historical Background, Literature Review on the Synthesis and Applicability of Azo-Dye Compounds: An Extensive Review. Adv. J. Chem. Sect. A, 7, 687-724 .
11.Han, H. J., & Gysi, A. P. (2024). UV-Vis spectrophotometric determination of rare earth elements (REE) speciation at near-neutral to alkaline pH. Part I: m-cresol purple properties from 25–75° C and Er hydrolysis. Dalton Transactions, 53(31), 13129-13141 .
12.Hassan, M. J. M., & Mizher, O. Q. (2018). New spectrophotometric estimation and cloud point extraction of cefdinir. Baghdad Science Journal, 15(4), 425–435. https://doi.org/10.21123/bsj.2018.15.4.0425.
13.Hassan, M. J. M., & Al-Rubaiawi, M. S. F. (2017). Cloud point extraction spectrophotometric method for determination of three types of cephalosporin via diazotization reactions with different reagents. Brazilian Journal of Analytical Chemistry, 4(17), 24–36.
14.Imran, A. M., Dhahir, S. A., & Muklive, A. J. (2024). Cloud point extraction method for spectrophotometric determination of 3-aminophenol in environmental samples. Indonesian Journal of Chemistry, 24(4), 1023–1033. https://doi.org/10.22146/ijc.89922.
15.Ji, Z., Li, X., Dong, Y., Wen, J., Feng, N., & Chen, Y. (2025). Immobilization of enzyme with MOF enhances colorimetric performance for fast, sensitive, and economical detection of chloramphenicol. Journal of Hazardous Materials, 139617 .
16.Hussein, A. A., & Mohammed, I. S. (2025). Spectrophotometric determination by azo coupling reaction of bisphenol A using benzidine reagent. Proceedings of the 13th International Conference on Applied Innovations in IT, 13(2), 595–606. https://doi.org/10.25673/120547 .

17.Mustafa, M. S., Mohammad, N. N., Radha, F. H., Kayani, K. F., Ghareeb, H. O., & Mohammed, S. J. (2024). Eco-friendly spectrophotometric methods for concurrent analysis of phenol, 2-aminophenol, and 4-aminophenol in ternary mixtures and water samples: assessment of environmental sustainability. RSC Advances, 14(23), 16045-16055 .

18.Oueslati, K., Lima, E. C., Naifar, A., Aouaini, F., Basha, B., Alrebdi, T., & Lamine, A. B. (2025). A combined experimental and theoretical investigation via statistical physics for adsorption evaluation of 3 aminophenol (AMP) onto the activated carbon derived from avocado seeds (ASAC) adsorbent. Adsorption, 31(2), 43 .

19.Poonguzhali, E., Kapoor, A., & Prabhakar, S. (2023). Membrane assisted process intensification and optimization for removal and recovery of phenol from industrial effluents. Separation and Purification Technology, 319, 124026 .

20.Roustaei, F., Baghdadi, M., Marjani, A., & Alimoradi, M. (2024). Spectrophotometric determination of phenol impurity in phenoxyethanol and phenol index of drinking water and municipal wastewater effluent after salting-out assisted liquid phase microextraction (SA-LPME). Heliyon, 10 .(٥)

21.Sahar, H., Gilani, M. R. H. S., Ansari, T. M., ul Ain, N., Maajeed, S., Zhao, J., Aqeel, M., & Xu, G. (2024). Electrochemical sensor of 3-aminophenol-formaldehyde polymer nanosphere/phosphotungstic acid nanocomposite modified electrode for nitrite detection. Environmental Engineering and Management Journal, 23(5), 983-990 .

22.Schram, J., Parrilla, M., Sleegers, N., Slosse, A., Van Durme, F., van Nuijs, A. L., & De Wael, K. (2024). Electrochemical classification of benzodiazepines: A comprehensive approach combining insights from voltammetry and liquid chromatography– mass spectrometry. Talanta, 279, 126623 .

23.Sengupta, S., & Das, P. (2022). Application of diazonium chemistry in purine modifications: A focused review. Journal of Heterocyclic Chemistry, 59(1), 5-21 .

24.Soliman, M. A., Pedersen, J. A., & Suffet, I. M. (2004). Rapid gas chromatography–mass spectrometry screening method for human pharmaceuticals, hormones, antioxidants and plasticizers in water. Journal of Chromatography A, 1029(1-2), 223-237 .

25.Tabaraki, R., & Heidarizadi, E. (2019). Spectrophotometric determination of phenol and chlorophenols by salting out assisted liquid-liquid extraction combined with dispersive liquid-liquid microextraction. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 215, 405-409 .

26.Wang, X., Lu, Z., Sun, W., Ye, S., & Tao, X. (2022). High-performance colorimetric immunoassay for determination of chloramphenicol using metal–organic framework-based hybrid composites with increased peroxidase activity. Microchimica Acta, 189(12), 484 .

27.Yadav, A., Basu, A., & Chakarbarti, A. (2019). Method for estimation of hippuric acid as a biomarker of toluene exposure in urine by high-performance liquid chromatography after extraction with ethyl acetate. Environmental Disease, 4(1), 17-22 .

28.Yang, H. J., Lim, S., Lee, D. H., Yun, C. I., & Kim, Y. J. (2024). Validation and measurement uncertainty of analytical methods for various azo dye adulterants in Curcuma longa L. Journal of Food Science, 89(9), 5993-6004 .

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