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Statistically Optimized Catalyst-Free Nitration of Salicylic Acid to 5-Nitrosalicylic Acid as a Key Mesalazine Precursor

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Abstract A statistically optimized, catalyst-free method was developed for the direct nitration of salicylic acid to produce 5-nitrosalicylic acid, an important precursor to the anti-inflammatory drug mesalazine. The proposed approach provides a direct alternative to the conventional industrial route, which involves multiple reaction steps and the formation of undesired isomers. Response surface methodology (RSM) was employed to investigate the effects of temperature, nitric acid concentration, reaction time, and the salicylic acid (SA) to nitric acid molar ratio on the synthesis process. Quadratic models with high goodness of fit ($R^2 > 0.99$) were developed, and numerical optimization was subsequently performed to determine the model predicted optimum conditions. The RSM-predicted optimum was obtained at a temperature of −4.90 °C, a nitric acid concentration of 80.60%, a reaction time of 188.41 min, and an SA:HNO_3 molar ratio of 1:10. Experimental validation under these conditions afforded a 5-nitrosalicylic acid yield of 48.9 ± 0.3% and a total isolated yield of 92.7 ± 0.4% (n = 3). The difference between the desired-product yield and the total isolated yield indicates moderate regioselectivity. Overall, the developed method enables direct, catalyst-free nitration of salicylic acid and provides a statistically optimized route to an important pharmaceutical precursor.

Keywords: 5-Nitrosalicylic acid, Mesalazine, Nitration, Salicylic acid, Response Surface Methodology, Ulcerative colitis.

1.Introduction

Mesalazine (5-aminosalicylic acid, 5-ASA) is a well-established first line therapy for mild to moderate ulcerative colitis and is widely used to induce and maintain remission [1, 2]. In 2007, the U.S. Food and Drug Administration (FDA) approved an extended-release formulation of mesalazine for the induction of remission in patients with active, mild-to-moderate ulcerative colitis [3]. Mesalazine has also been investigated for its potential role in reducing the risk of colorectal cancer in patients with inflammatory bowel diseases (IBD), although the evidence for a chemopreventive effect remains inconclusive. Its therapeutic effects have been associated, in part, with its anti-inflammatory and antioxidant properties, including the inhibition of inflammatory pathways and scavenging of reactive oxygen species [4, 5].

Several synthetic methods to mesalazine have been reported since its early synthesis by electrolysis of nitrobenzoic acid in the presence of sulfuric acid [6]. Most approaches involve the reduction of 5-nitrosalicylic acid using different reducing agents. For example, Golinoff reported the use of tin powder in hydrochloric acid in 1928, while Popov employed catalytic hydrogenation over Ni [7]. Owing to its moderate reaction conditions and acceptable yield, Popov's method provided a basis for subsequent studies aimed at process optimization.

Other approaches have used different precursors. Reduction of 5-phenylazosalicylic acids afforded mesalazine with in-situ formation of aniline as a by-product [8]. Mesalazine has also been synthesized from sulfasalazine in water as a solvent [9]. The electrochemical reduction of 5-nitrosalicylic acid to mesalazine was demonstrated using a copper electrode, affording a yield of approximately 90% [10]. The process is sensitive to reaction conditions, including temperature, electrolyte concentration (e.g., KOH and $NaHCO_3$), and electrode material, with Cu reported to perform better than Ni [11]. The reduction of 5-nitrosalicylic acid to mesalazine by hydrogenation over Raney Ni was compared with hydrazine reduction. Owing to the high toxicity of hydrazine, hydrogenation was considered a more suitable approach and demonstrated potential for industrial application [12]. A scalable, high-yielding synthesis of mesalazine from chloronitrobenzoic acid was also reported through a two-step process, affording 82% yield with 99% purity and demonstrating its suitability for scale-up [13]. A further method involved the gas-phase catalytic carboxylation of *p*-aminophenol with CO_2 under elevated temperature and pressure. The process uses a solid catalyst and a basic compound, with temperature and pressure as key operating parameters, and was reported to be suitable for industrial-scale production [14]. Recently, mesalazine has also been synthesized by photocatalytic reduction of 5-nitrosalicylic acid using TiO_2, providing an alternative reduction strategy [15]. A four-step synthesis from nitrobenzene was also reported, in which Ni, Pd, and organometallic catalysts were evaluated for their effects on product yield and selectivity [16]. Overall, the reported methods for mesalazine synthesis employ a variety of precursors, catalysts, and reaction conditions, with some involving multiple steps or relatively demanding operating conditions [17]. These factors can increase process complexity and limit the practical efficiency of the synthesis. Therefore, continued development of simpler and more efficient synthetic methods remains of interest, particularly those based on readily available and cost effective starting materials such as salicylic acid. Nitration is one of the most widely used chemical transformations in industry [17]. Nitroaromatic compounds produced through nitration serve as important intermediates in the manufacture of polymers, dyes, pharmaceuticals, fertilizers, explosives, and other chemicals [18]. Over the past decades, various nitration strategies have been developed to address the limitations of

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conventional processes. Industrial nitration commonly relies on mixed-acid systems, typically involving nitric and sulfuric acids [7]. Although widely established, these systems are associated with limited process selectivity and the generation of substantial acidic waste. Alternative nitrating systems, including nitrate salts, dinitrogen pentoxide (N_2O_5), and supported nitrating agents, have therefore been investigated, each with distinct advantages and limitations [6]. Additionally**,** heterogeneous catalytic systems based on zeolites and alumina–silica matrices have been developed to improve selectivity and process efficiency [7,8]. Among nitrating agents, nitric acid is one of the most widely available and commonly used reagents and is conventionally employed with H_2SO_4 as a catalyst. Alternative systems have also been investigated for the nitration of salicylic acid. For example, HNO_3/HOAc has been employed for the synthesis of 5-nitrosalicylic acid [19]. A further approach demonstrated the nitration of phenolic compounds under milder and potentially safer conditions, with calcium salts formed as useful by-products for agricultural applications [20]. A mild nitration approach based on $NaNO_3$ and benzyltriphenylphosphonium peroxodisulfate (BTPPPDS) was reported for aromatic compounds in acetonitrile, using a 1:1:1.2 molar ratio of substrate/BTPPPDS/$NaNO_3$ and providing moderate to high yields. Subsequently, transition-metal complexes were introduced to promote nitration with 69% HNO_3. Several complexes, including Ni-, Co-, Cu-, Mn-, and Hg-based systems, were demonstrated to effectively facilitate the reaction [21]. Conventional nitration using concentrated HNO_3/H_2SO_4 is often associated with excessive acidic waste, over nitration, and limited selectivity. To overcome these limitations, Werner-type transition metal complexes were reported as effective promoters for aromatic nitration under relatively mild conditions. Their proposed role involves coordination of HNO_3 to the metal center, facilitating nitronium ion generation and subsequent electrophilic aromatic substitution. The heterogeneous nature of these systems also allows relatively convenient catalyst recovery [22]. In view of the established importance of 5-nitrosalicylic acid (5-NSA) as a key precursor in the synthesis of mesalazine, the development of efficient and well-controlled methods for its preparation remains an important research target. In this study, a direct and catalyst-free nitration of salicylic acid is investigated as a synthetic approach to 5-NSA, without the use of metal catalysts or additional catalytic acids. A statistically designed experimental approach based on response surface methodology (RSM) is employed to systematically evaluate the effects and interactions of the principal reaction variables and to establish optimized conditions for 5-NSA formation. The study aims to provide a practical and experimentally validated nitration process that combines regioselective formation of 5-NSA with controlled reaction conditions and efficient use of the starting material.

2.Experimental

All chemicals, including salicylic acid, ethanol, and HNO_3 (100%) were purchased from Merck and used without more purification. Concentrated nitric acid was diluted with distilled water to the required concentrations before use. The characterization of the obtained product was made using the following equipment: The Fourier transform infrared (FT-IR) spectra were run on a Nicolet 800-American spectrometer; nuclear magnetic resonance (NMR) analysis was recorded on a Bruker Avance spectrometer at 500 and 125 MHz, respectively. Chemical shifts (δ) are given in ppm from tetramethylsilane (TMS) as an internal standard. Differential scanning calorimetry (DSC) analysis was performed using a Mettler Toledo TGA/DSC instrument in aluminum crucibles under an argon atmosphere at a heating rate of 10 °C min^{-1}. The sample mass was 9.2 mg. The chromatographic measurements of 5-nitrosalicylic acid were performed on an HPLC system (Waters 600 HPLC system, Madrid, Spain) with UV–Vis detector (wavelength = 210 nm).

General Procedure: Synthesis of 5-Nitrosalicylic Acid

A 50 mL double-walled reactor equipped with a magnetic stirrer, a thermometer, and a cooling chiller was used. Nitric acid (85%; 7.77 mL, 0.18 mol) was charged into the reactor. Subsequently, salicylic acid (2.5 g, 18 mmol) was added to the mixture in four portions over a period of one hour, while the temperature was maintained at -5 °C. After 2 hours, the reaction mixture was filtered under vacuum and washed three times with 15 mL of cold water. After drying in an oven at 50°C, the crude product was recrystallized from 70% ethanol, and 5-nitrosalicylic acid was obtained as a light-yellow solid in a 49% yield with a melting point of 230-235 °C.

The scale-up experiment was carried out using a 2 L glass double-walled reactor equipped with a mechanical stirrer and connected to a chiller for temperature control. Initially, 201.5 g of distilled water was charged into the reactor, followed by the addition of 1140.50 g of 100% nitric acid, corresponding to a 10-fold molar excess relative to salicylic acid. The addition of water was performed to adjust the nitric acid concentration to 85%. The resulting nitric acid solution was cooled and maintained at -5 °C using the chiller. Subsequently, 250 g of salicylic acid was added gradually to the 85% nitric acid in eight portions. The salicylic acid was divided into four portions of 25 g, two portions of 30 g, and two portions of 45 g. The portions were added sequentially while maintaining the temperature of the nitration mixture below +5 °C. After each addition, sufficient time was allowed for the temperature of the reaction mixture to decrease to approximately -5 °C before the next portion was introduced. The gradual addition of salicylic acid required approximately 1 h. After the final portion had been added, the reaction mixture was stirred for an additional 2 h at -5 °C to allow completion of the nitration reaction. The reaction mixture was stirred at 150 rpm throughout the reaction. The resulting mixture was then filtered, and the collected solid was washed three times with 200 mL portions of cold distilled water. The washed solid was dried in an oven at 50 °C. After recrystallization of the crude product using 70% ethanol (the volume of ethanol used for recrystallization was 5 mL per gram of salicylic acid)**,** pure 5-nitrosalicylic acid was obtained in a 49% yield.

FT-IR (KBr, cm^{-1}): 3400-2400 (br, O–H, phenolic and carboxylic acid), 1670 (C=O, -carboxylic acid), 1620, 1448 (C=C, phenyl), 1515, 1339 (s, -N-O), 1250 (C-O, phenolic). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 13.02 (s, 2H, -OH, phenolic and carboxylic acid), 8.47 (1H, d, *J*= 3.0 Hz, Ar-H), 8.25 (1H, dd, *J*= 9.3 Hz, 2.9 Hz, Ar-H), 7.07 (1H, d, *J*= 9.1 Hz, Ar-H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 170.0, 165.8, 139.1, 129.9, 126.5, 118.4, 113.7.

Validation experiment under optimized conditions

For validation of the RSM-predicted optimum, the reaction was repeated under the predicted conditions obtained from Design-Expert software (temperature = −4.90 °C, HNO_3 concentration = 80.60%, reaction time = 188.41 min, and SA:HNO_3 molar ratio = 1:10). The reaction procedure, isolation, and purification steps were performed according to the general procedure described above. The experiment was carried out in triplicate, and the individual replicate values are provided in Table 1. The obtained experimental responses were compared with the model-predicted values.

Experimental Design and Statistical Analysis

To optimize the nitration process and better understand the

Table 1. Individual replicate values of the validation experiment under the RSM-predicted optimum conditions.

Replicate	5-Nitrosalicylic acid yield (%)	Total isolated yield (%)
1	48.6	92.3
2	49.2	93.1
3	48.9	92.7
Mean ± SD	48.9 ± 0.3	92.7 ± 0.4

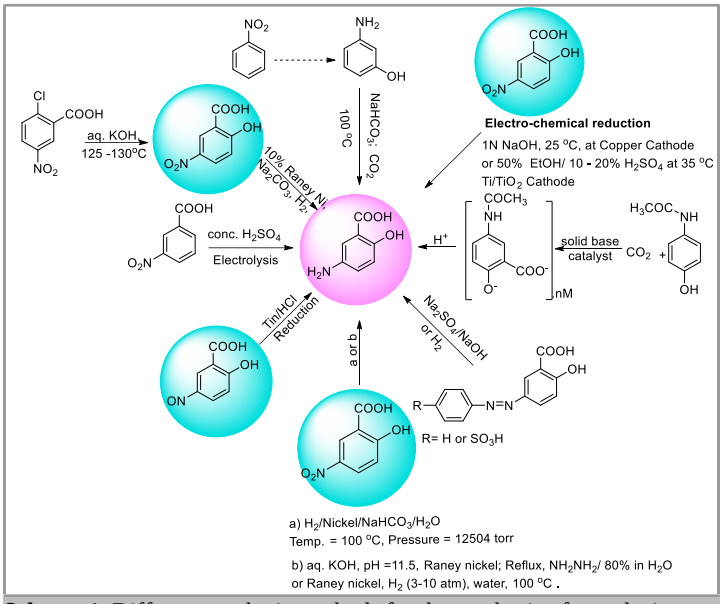
interactions among the process parameters, response surface methodology (RSM) was employed. The experimental design was established using an I-optimal (coordinate exchange) algorithm implemented in Design-Expert® software (version 12.0.3.0, Stat-Ease, Inc., Minneapolis, USA). This type of design is well suited for fitting a second-order polynomial model and can efficiently accommodate factors integer and non-integer values.

The experiment was designed to investigate the effects of four independent variables; A) temperature (−5 to +5 °C), B) nitric acid concentration (80 to 100%), C) reaction time (60 to 210 min), and D) salicylic acid to nitric acid molar ratio (1:3, 1:5, 1:10, and 1:15). This factor was modelled categorically because the selected stoichiometric ratios represent distinct reaction conditions rather than equally spaced numerical levels. For statistical modelling, factor D was coded as a four-level categorical variable in Design-Expert software. The categorical factor contributed three degrees of freedom corresponding to the four tested stoichiometric levels (1:3, 1:5, 1:10, and 1:15). Accordingly, no quadratic term (D^2) was considered for this factor, whereas interactions involving factor D were evaluated according to the selected model hierarchy. The results are shown in Table 2.

The experimental design consisted of a total of 32 runs, including replicates to allow estimation of pure error. The adequacy of the developed models was evaluated using analysis of variance (ANOVA), lack of fit tests, and diagnostic plots. Statistical significance was evaluated at an α-level of 0.05. A numerical optimization method based on desirability function was employed to simultaneously optimize both responses (Table 2).

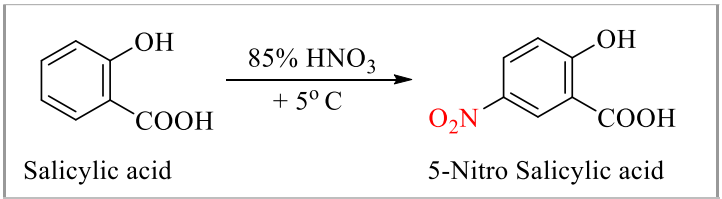
3.Results and Discussion

Scheme 1 summarizes representative synthetic routes to mesalazine, with particular emphasis on the salicylic acid derivatives employed as key intermediates in these approaches. They illustrate the use of appropriately functionalized salicylic acid derivatives as important intermediates in the preparation of 5-aminosalicylic acid. Among these intermediates, 5-nitrosalicylic acid is particularly relevant because its nitro group can be readily transformed into the corresponding amino functionality. Accordingly, the synthesis of 5-NSA represents an important step in the preparation of mesalazine and provides a suitable basis for evaluating alternative nitration strategies. The conventional synthetic routes for the nitration of salicylic acid to produce 5-nitrosalicylic acid subject to several limitations including poor regioselectivity and low yields, which result in the formation of undesired isomers and side products [9]. Moreover, various estabilished nitration approaches require complex catalysts and involve time-consuming and expensive procedures. These limitations not only increase production costs but also raise concerns about the environmental sustainability of these methods due to the generation of significant amounts of chemical waste. Therefore, the development of a simple, efficient and cost effective



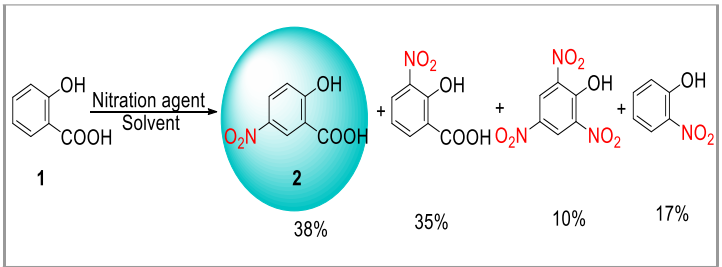
Scheme 1. Different synthetic methods for the synthesis of mesalazine.

nitration process for the direct synthesis of 5-NSA from salicylic acid is highly desirable (Scheme 2).



Scheme 2. Synthesis of 5-nitrosalicylic acid by nitration reaction of salicylic acid.

The nitration of salicylic acid is complicated from an organic chemistry perspective because the molecule contains two functional substituents, namely carboxylic acid and phenolic hydroxyl groups, which exert opposing electronic effects during electrophilic aromatic substitution. While the hydroxyl group is activating and directs substitution toward the *ortho* and para positions, the carboxylic acid group is deactivating and directs substitution to *meta* positions. These opposing electronic effects, together with possible steric effects, can lead to the formation of multiple nitration isomers. As illustrated in Scheme 3, this not only complicates the control of regioselectivity but may also reduce the yield of the desired 5-nitro isomer.



Scheme 3. Formation of different nitrosalicylic acid isomers during the nitration of salicylic acid.

In the pharmaceutical sector, both commercial and quality considerations influence the selection of methods and precursors.

Table 2. Design matrix

Run	Build Type	Factor 1 A: Temp	Factor 2 B: HNO_3	Factor 3 C: Time	Factor 4 D: Ratio	Response 1 5-nitrosalicylic acid (%)	Response 2 total (%)
1	Model	0	80	210	1:3	35.1	70.6
2	Model	5	80	210	1:5	28.3	61.6
3	Replicate	0	80	210	1:3	36.3	72.8
4	Replicate	0	90	210	1:10	39	76.9
5	Model	5	100	135	1:10	3.6	34.1
6	Model	5	90	60	1:3	2.3	33.8
7	Lack of Fit	-5	90	60	1:3	24.1	60
8	Model	-5	80	210	1:15	47.5	87.5
9	Model	0	80	135	1:5	33.2	73.2
10	Replicate	5	90	210	1:15	23.8	57
11	Model	5	80	60	1:15	18.5	51
12	Model	0	80	60	1:10	32.6	75.9
13	Model	0	90	210	1:10	40.4	79.5
14	Model	0	100	135	1:5	12.1	41.1
15	Lack of Fit	-5	90	135	1:10	41.2	83.3
16	Replicate	0	100	135	1:5	13.3	43.4
17	Model	5	90	60	1:5	3.9	32.4
18	Lack of Fit	-5	90	60	1:15	29.3	66.1
19	Lack of Fit	-5	100	60	1:3	7	36.7
20	Model	-5	100	60	1:10	15.7	54.8
21	Model	-5	100	210	1:15	26.6	61.5
22	Model	5	90	210	1:15	24.4	58.6
23	Model	5	100	210	1:3	0.5	30
24	Model	-5	90	210	1:5	38.9	77.6
25	Model	0	100	60	1:15	5.2	30.9
26	Model	5	80	210	1:10	49	93
27	Model	-5	90	135	1:15	37	77.8
28	Model	-5	100	135	1:3	18.8	50.1
29	Model	-5	100	60	1:5	11.9	40.3
30	Lack of Fit	5	80	135	1:10	29.4	66.7
31	Replicate	0	80	135	1:5	34.1	75.8
32	Model	-5	80	60	1:3	31.4	69.3

Accordingly, in this study, both homogeneous and heterogeneous catalysts were avoided due to economic considerations. In addition, to reduce the environmental impact and simplify the process, no additional solvent was used.

The efficiency of the nitration process is influenced by four key parameters: nitric acid concentration, the molar ratio of SA: HNO_3, temperature, and reaction time. First, to determine the appropriate temperature range, the nitration of salicylic acid was performed at -10, -5, 0, 5, and 10 °C (Table 3, Entries 1-5). As the temperature increased, decarboxylation became more pronounced, resulting in a lower yield (Table 3, Entry 5). At temperatures of -5 °C, the total yield was 67%, while the yield of the desired 5-nitrosalicylic acid was 22% (Table 3, Entry 2). In contrast, at excessively low temperatures, the reaction rate decreased considerably, resulting in limited reaction progress.

Moreover, the concentration of HNO_3 and its stoichiometric ratios significantly affect the reaction yield. To optimize the acid concentration, the nitration reaction was carried out at concentrations of 100%, 95%, 90%, 85%, and 80% (Table 3, Entries 2, 6-9). At higher acid concentrations, decarboxylation reaction becomes more pronounced, leading to formation of phenolic compounds and carbon dioxide gas [23]. The higher temperatures associated with increased acid concentrations may also promote the formation of nitrogen oxide vapors. Consequently, the yield of 5-nitrosalicylic acid decreases (Table 3, Entry 2). The highest yield was obtained using 85% HNO_3 (Table 3, Entry 8).

Furthermore, SA:HNO_3 molar ratio was optimized by 1:3, 1:5, 1:10, and 1:15 (Table 3, Entries 8, 10-12). Among the conditions evaluated, the highest yield in the preliminary OFAT experiments was obtained at an SA:HNO_3 molar ratio of 1:10 (Table 3, Entry 11). Subsequently the nitration reaction was performed at -5 °C using 85% HNO_3 and an SA: HNO_3 molar ratio of 1:10. The optimal reaction time was determined about three hours (Table 3, Entries 11, 13-16). In addition to temperature, acid molarity, SA:HNO_3 molar ratio, and reaction time, the mixing technique and stirring speed were also considered as key variables. Two conventional mixing techniques were investigated: (1) dissolving salicylic acid in an organic solvent such as HOAc, CH_2Cl_2, or MeOH, followed by the gradual addition of HNO_3; and (2) charging the reactor with 85% HNO_3, cooling it to the desired temperature, and then adding powdered salicylic acid. Laboratory experiments demonstrated that although the first approach provides greater control over the reaction temperature, it is less favorable due to its lower yield and the use of organic solvents, which introduce environmental and practical concerns. In contrast, the second approach involving the direct addition of salicylic acid to HNO_3, proved to be more practical and effective, affording the highest product yield. This approach not only simplifies the experimental procedure but also provides economic and environmental benefits by eliminating the need for organic solvents.

Preliminary Investigation and Factor Effects

The experiments summarized in Table 3 represent a preliminary one-factor-at-a-time (OFAT) screening stage. These experiments were conducted separately to evaluate the individual effects of temperature, nitric acid concentration, reaction time, and reagent ratio and to establish suitable experimental ranges for the subsequent response surface methodology study. Because the OFAT approach does not allow the evaluation of interactions among variables, a separate 32-run I-optimal design was subsequently employed. The OFAT experiments were not included as observations in fitting the subsequent response surface model.

Table 3. Optimization of salicylic acid nitration with HNO_3.

Entry	Temperature (°C)	HNO_3 concentration (%)	Molar Ratio HNO_3: SA	Time (min)	Total Yield (%)	Yield (%) (5-nitrosalicylic acid)
1	-10	100	1:5	60	62	20
2	-5	100	1:5	60	67	22
3	0	100	1:5	60	60	10
4	5	100	1:5	60	13	-
5	10	100	1:5	60	8	-
6	-5	95	1:5	60	72	28
7	-5	90	1:5	60	76	31
8	-5	85	1:5	60	82	38
9	-5	80	1:5	60	63	21
10	-5	85	1:3	60	76	35
11	-5	85	1:10	60	88	41
12	-5	85	1:15	60	80	39
13	-5	85	1:10	90	81	40
14	-5	85	1:10	140	89	45
15	-5	85	1:10	180	93	49
16	-5	85	1:10	210	93	49

Therefore, a full scale analysis based on response surface methodology was subsequently performed. The analysis of variance (ANOVA) results for the developed quadratic models (Table 4) demonstrated that the models were statistically significant for both responses (p-value < 0.0001), whereas the lack of fit was not statistically significant (p-value > 0.05).

The model statistics demonstrated an excellent fit and strong predictive capability. The factor D (molar ratio) showed a statistically significant effect, indicating that the stoichiometric ratio strongly influenced the reaction outcome. The negative coefficients for temperature and acid concentration, suggest that lower levels of these parameters favour the suppression of decarboxylation reactions and other side reactions. In contrast, the positive coefficient for reaction time indicates a favourable effect on the response, suggesting that a longer reaction time is desirable under the investigated conditions.

The positive coefficients of the AB, AC, and BC interaction terms reflect significant interactions among the investigated factors, highlighting the complexity of the process.

Table 4. Analysis of variance (ANOVA) for the quadratic models.

Source	5-Nitro (F-value)	5-Nitro (p-value)	Total (F-value)	Total (p-value)
Model	242.91	<0.0001	264.17	<0.0001
A-Temp	1378.67	<0.0001	1472.70	<0.0001
B-HNO_3	1785.95	<0.0001	1952.61	<0.0001
C-Time	777.67	<0.0001	531.51	<0.0001
D-Ratio	92.36	<0.0001	129.78	<0.0001
AB	14.52	0.0034	7.37	0.0218
AC	8.44	0.0157	11.01	0.0078
BC	8.21	0.0168	22.33	0.0008
A^2	36.36	0.0001	37.76	0.0001
B^2	86.32	<0.0001	90.74	<0.0001
C^2	18.03	0.0017	28.90	0.0003

*Model Statistics: For 5-Nitro: R^2=0.9980, Adj R^2= 0.9939, Pred R^2= 0.9398. For Total: R^2= 0.9981, Adj R^2= 0.9940, Pred R^2= 0.9817.

Visualization of Factor Interactions and Response Surfaces

The perturbations plot (Figure 1A) provides a clear visual representation of the individual effects of the investigated parameters on the yield of 5-nitrosalicylic acid. The pronounced negative slopes observed around the centers point indicate a strong inhibitory effect of the corresponding parameter with increasing parameter level, whereas less steep slopes suggest weaker effects. Temperature (A) and HNO_3 concentration (B) exhibit relatively strong inhibitory effects, with the yield decreasing as their levels increase.This three-dimensional response surface plot provides a clear representation of the interaction between temperature and HNO_3 concentration. The concave shape of the response surface indicates that the maximum yield is located near the region corresponding to low temperatures and low HNO_3 concentration. This observation further indicates that increasing either parameter beyond its optimal range adversely affects the yield. Figure 2 illustrates the interaction between HNO_3 concentration and reaction time. The corresponding two-dimensional graphical representation indicates that at low HNO_3 concentrations, increasing the reaction time has a positive effect on the total yield. In contrast, at high HNO_3 concentrations, increasing the reaction time exerts a negative effect on the total yield. Figure 2 also presents the three-dimensional response surface plot illustrating the interaction between reaction time and temperature with respect to the total yield. As shown in Figure 2B, when the temperature is maintained at -5 °C, which corresponds to figure 1 the optimal temperature level, increasing the reaction time has a positive effect on the yield. However, at higher temperatures, the effect of increasing the reaction time becomes negative.

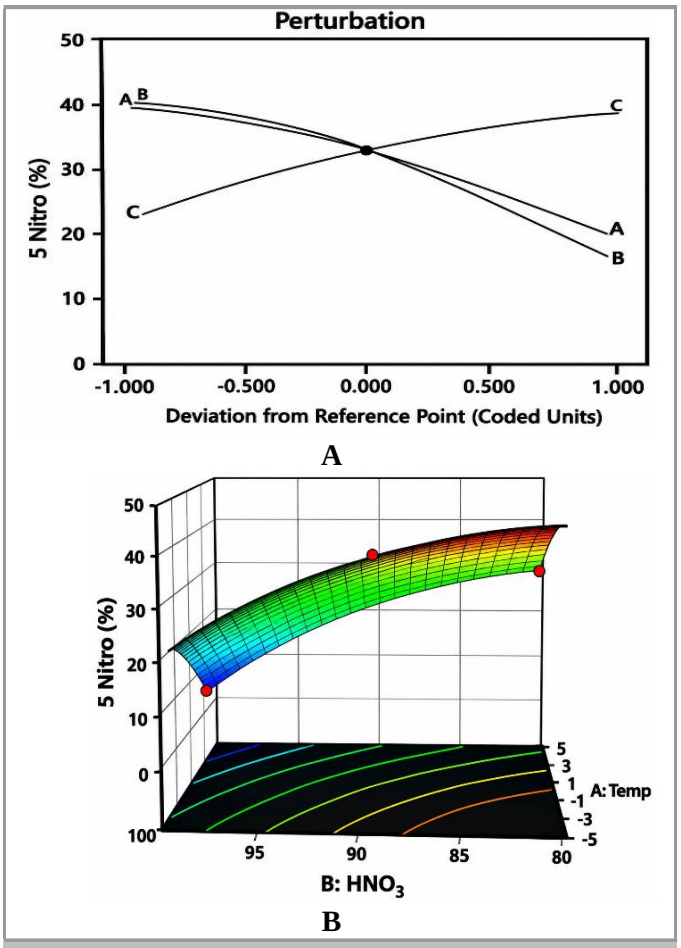


Figure 1. (A) Perturbation plot illustrating the individual effects of the investigated factors on the yield of 5-nitrosalicylic acid. (B) Three-dimensional response surface plot illustrating the combined effects of temperature and HNO_3 concentration on the yield of 5-nitrosalicylic acid at a reaction time of 135 min and an SA:HNO_3 molar ratio of 1:10

Numerical Optimization and Model Validation

Numerical optimization based on the desirability function approach was performed using the fitted response surface models to identify the most desirable operating condition within the investigated experimental design space. The optimization procedure simultaneously considered the predicted yield of 5-nitrosalicylic acid and the total isolated yield as response variables.

The desirability ramp plot (Figure 3A) illustrates the contribution of each factor toward achieving the maximum overall desirability. The obtained solution corresponded to a temperature of −4.90 °C, HNO_3 concentration of 80.60%, reaction time of 188.41 min, and an SA:HNO_3 molar ratio of 1:10. Under these conditions, the model predicted a 5-nitrosalicylic acid yield of 48.01% and a total yield of 93.01%, with an overall desirability value of 1.000.

The three-dimensional response surface plot (Figure 3B) demonstrates the region of the experimental design space where the response criteria were simultaneously satisfied. The predicted optimum region was located at low temperature and relatively low nitric acid concentration, confirming the significant influence of these factors on product formation and side-reaction suppression.

Although the preliminary OFAT experiment at −5 °C, 85% HNO_3, SA:HNO_3 ratio of 1:10, and 180 min provided a higher

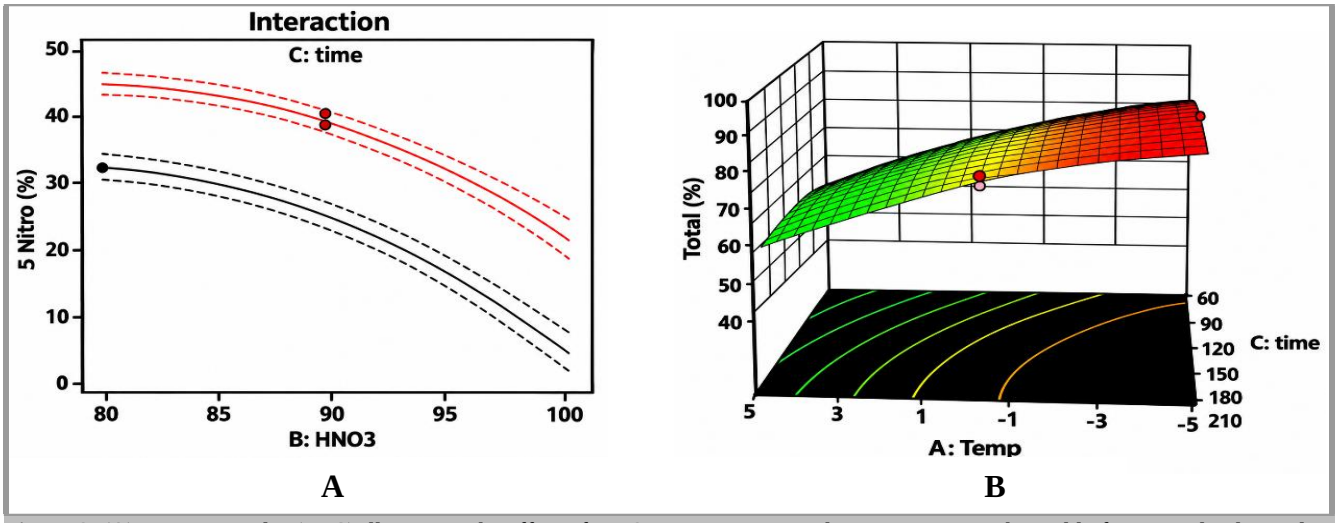


Figure 2. (A) Interaction plot (B×C) illustrating the effect of HNO_3 concentration and reaction time on the yield of 5-nitrosalicylic acid (temperature = -5 °C, SA:HNO_3 molar ratio = 1:10). (B) Three-dimensional response surface plot illustrating the combined effects of reaction time and temperature on total yield (HNO_3= 80.60%, SA: HNO_3 molar ratio = 1:10).

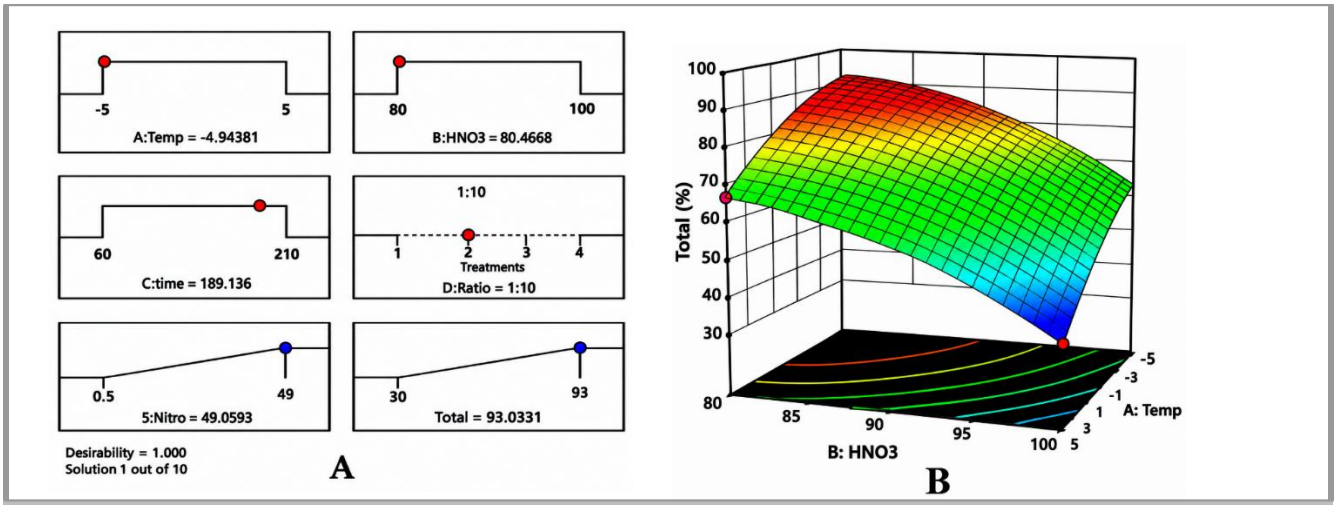


Figure 3. (A) Desirability ramp diagram for numerical optimization. (B) Three-dimensional response surface plot showing the predicted total yield as a function of temperature and HNO_3 concentration, highlighting the RSM model predicted optimum region (reaction time = 188.41 min, SA:HNO_3 molar ratio = 1:10) .

Table 5. RSM model predicted optimum conditions and validation experiment results.

Condition	Optimal Value	Predicted 5-nitro (%)	Predicted Total (%)	Actual 5-nitro (%)	Actual Total (%)
Temperature	−4.90 °C	-	-	-	-
HNO_3 Concentration	80.60%	-	-	-	-
Time	188.41 min	49.01	93.01	48.9±0.3	92.7±0.4
Molar Ratio	1:10	-	-	-	-
Temperature	−5 °C	-	-	-	-

The overlay plot (Figure 3B) graphically defines the "sweet spot" where both response criteria are met. A verification experiment conducted at the recommended optimum yielded 48. 9 ± 0.3% of 5-nitrosalicylic acid and 92.7 ± 0.4% total isolated yield, showing good agreement between the predicted and experimental responses.

experimentally observed yield, it should be noted that the RSM model-predicted optimum represents the optimum solution within the fitted response surface model and should not be interpreted as the highest experimentally observed yield among all experiments performed in this study. The preliminary OFAT experiments presented in Table 3 were conducted independently before RSM modelling and included conditions that resulted in slightly higher experimentally observed yields. The OFAT experiment and the RSM validation experiment were performed using the same reactants, analytical procedures, and response definitions. Therefore, the observed difference arises from the optimization strategy rather than from differences in experimental batches or analytical methods.

A validation experiment was subsequently performed under the predicted optimum conditions to evaluate the accuracy of the model prediction (Table 5). The experimental validation resulted in 48.9 ± 0.3% yield of 5-nitrosalicylic acid and 92.7 ± 0.4% total isolated yield (n = 3). The relative prediction errors for 5-nitrosalicylic acid yield and total yield were calculated as 0.22% and 0.33%, respectively. As shown in Table 6, the reported nitration methods for preparing 5-nitrosalicylic acid from salicylic acid differ in reaction medium, acid composition, temperature, reaction time, and reactor configuration. The differences in the reported reaction conditions also highlight the importance of these parameters in the nitration process. In the present study, response surface methodology was used to examine these factors systematically.

As summarized in Table 6, several reported methods provide yields above 80%, but these approaches often involve organic solvents, strong-acid mixtures, metal-containing reagents, microwave irradiation, elevated temperature or pressure, or specialized reaction systems. Recent continuous-flow and microreactor approaches offer advantages such as rapid mixing, efficient heat transfer, short residence times, but may require specialized equipment and precise process control. In addition, precipitation of 5-NSA can present a risk of microchannel blockage, particularly in continuous-flow systems [40-42].

In comparison, the present method employs concentrated nitric acid without an added catalyst or external reaction solvent under controlled low-temperature conditions. Although its 5-NSA yield is lower than that of several reported methods, the reduced number of reaction components simplifies the reaction system and downstream handling.

Overall, the literature comparison indicates considerable variation among the reported methods in terms of yield, reaction time, process complexity, reagent and solvent requirements. Accordingly, the main contribution of the present work lies in integrating a catalyst-free, solvent-minimized nitration system with statistical optimization and a process-oriented evaluation, rather than focusing solely on maximizing the yield of 5-NSA.

Characterization of the Product

After the separation and purification of the product, 5-nitrosalicylic acid was characterized using a range of complementary analytical techniques. The FT-IR spectrum was consistent with the expected structure of 5-nitrosalicylic acid [43]. The characteristic absorption bands were assigned as follows. The sharp peak at 3444 cm^{-1} was attributed to the O-H stretching vibration of phenolic group. The broad absorption band observed in the 2400-3400 cm^{-1} was assigned to the hydrogen-bonded O–H stretching vibration of the carboxylic acid group. A strong absorption peak at 1670 cm^{-1} was attributed to the C=O stretching vibration of the carboxylic acid group. The characteristic skeletal vibrations of the aromatic ring were observed at 1625 cm^{-1} and 1448 cm^{-1}. The asymmetric and symmetric stretching vibrations of the nitro group (–NO_2) were observed at 1515 cm^{-1} and 1339 cm^{-1}, respectively.

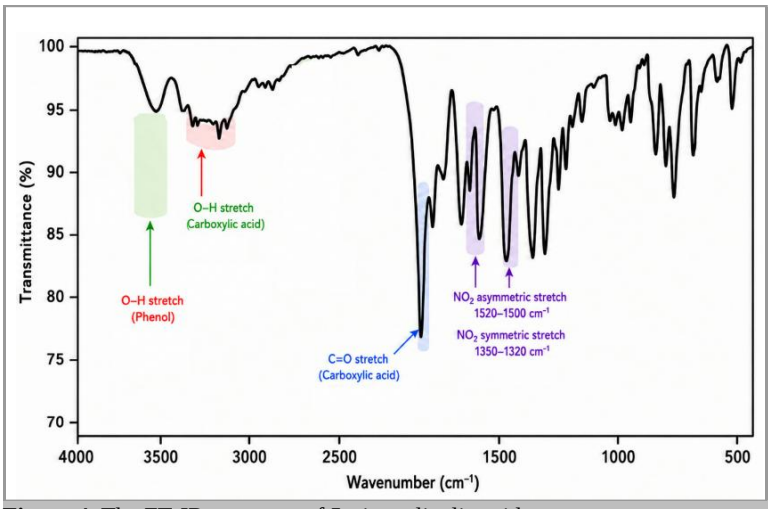


Figure 4. The FT-IR spectrum of 5-nitrosalicylic acid.

The ^{1}H-NMR spectrum of 5-nitrosalicylic acid showed characteristic resonances corresponding to the aromatic protons and the acidic proton. A downfield signal at δ 13.02 ppm was assigned to the carboxylic acid proton (–COOH), which appears at a relatively high chemical shift because of the strong deshielding effect of the carbonyl group and intramolecular hydrogen bonding. The aromatic region exhibits three distinct proton resonances, consistent with the 1,2,5-trisubstituted benzene ring. The signal at δ 8.47 ppm is assigned to H-6, which is expected to resonate at the lowest field because it is positioned between the electron-withdrawing carboxylic acid and nitro groups. The H-4 proton resonates at δ 8.25 ppm and is influenced by the neighboring nitro substituent, with coupling to the adjacent aromatic protons, whereas H-3 is affected by both the hydroxyl and nitro substituents and couples primarily with H-4. The most upfield aromatic resonance, observed at δ 7.02 ppm, is assigned to H-3, which is located *ortho* to the hydroxyl (–OH) group. H-3 primarily couples with the adjacent H-4 proton and consequently appears as a doublet. Overall, the observed chemical shifts and coupling patterns are in good agreement with the proposed structure of 5-nitrosalicylic acid [40].

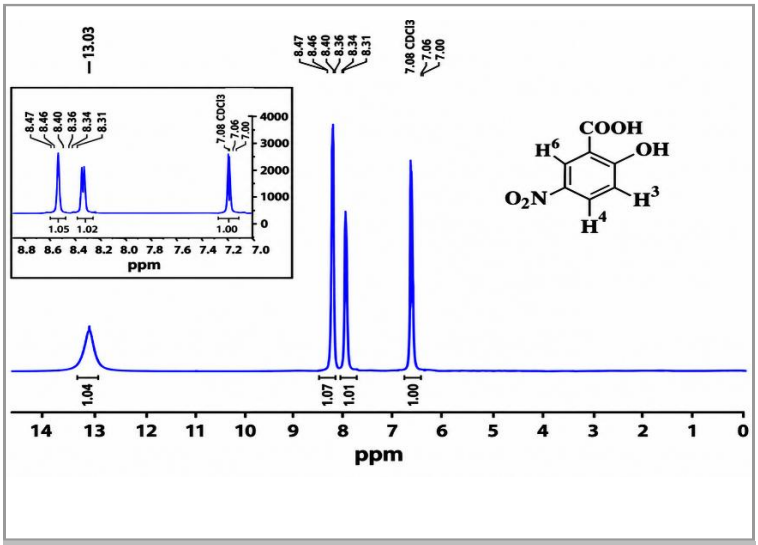


Figure 5. The ^{1}H-NMR spectrum of 5-nitrosalicylic acid in $CDCl_3$.

Table 6. Qualitative comparison of the economic and environmental aspects of the present approach with previously reported methods for the nitration of salicylic acid.

No.	Reagents and reaction medium	Reaction conditions	5-NSA yield (%)	Process characteristics and environmental–economic considerations	Ref.
1	Nitric acid, sodium dodecyl sulfate, acetonitrile	2.5 h, 25 °C	89	Use of a micellar medium and an organic solvent; although a high yield is achieved, the consumption of solvent and auxiliary additives should be considered in the overall process assessment.	[24]
2	Sulfuric acid, nitric acid, acetic acid	45 s, 120 °C, 0.4 MPa	88	Very short reaction time; however, the elevated temperature and pressure, together with the simultaneous use of strong acids, increase the energy demand and safety requirements of the process.	[25]
3	Copper(II) tetraammine sulfate, nitric acid, dichloromethane	2.5 h, 20 °C	86	High yield obtained using a metal-based catalyst and an organic solvent; the generation and management of metal- and solvent-containing waste streams should be taken into consideration.	[22]
4	Calcium nitrate, acetic acid	1 min, microwave irradiation	84	Very short reaction time with microwave heating; however, energy consumption and product isolation should be considered for a comprehensive process comparison.	[26]
5	Sodium nitrate, benzyltriphenylphosphonium peroxydisulfate, acetonitrile	65 h under reflux	70	Long reaction time and the use of multiple reagents and an organic solvent may increase the energy and auxiliary material requirements of the process.	[21]
6	Nitric acid, acetic acid	60 °C	67	Relatively simple reaction system; however, the use of acid and an organic solvent, as well as the need to separate by-products, should be considered in the overall process assessment.	[27]
7	Sulfuric acid, 44% nitric acid	0–7 °C	-	Use of a strongly acidic medium; quantitative yield data were not reported.	[28]
8	Lead(II) nitrate, glacial acetic acid	0 °C	-	Use of a lead-containing reagent; the management of heavy-metal-containing waste is an important environmental consideration.	[29]
9	Nitrosyl sulfuric acid, nitric acid	30 °C	-	Use of strong acidic reagents; quantitative data for the yield of 5-NSA were not reported.	[30]
10	Nitrosyl sulfuric acid, nitric acid	20 °C	-	Acidic nitration system; quantitative information on the 5-NSA yield was not provided in the source considered in the table.	[19]
11	70% nitric acid, water	Heating	-	Simple system in terms of the number of reaction components; however, no quantitative yield of 5-NSA was reported.	[31]
12	Nitric acid, chloroform	-	99	Very high yield; however, the use of chloroform as an organic solvent is relevant from both environmental and solvent-management perspectives.	[32]
13	Sulfuric acid, water, sodium nitrite	50 °C	56	Use of an acidic medium and nitrite; moderate yield and the formation of nitrogen-containing species in the side stream are notable considerations.	[33]
14	65% nitric acid, acetic acid	0 °C	-	Low-temperature nitration; quantitative yield data for 5-NSA were not reported.	[34]

15	Zinc nitrate, dinitrogen tetroxide, dichloromethane	5 min, 20 ℃	80	Short reaction time; however, the use of specialized reagents and an organic solvent introduces additional considerations related to reagent preparation, safety, and waste management.	[35]
16	Nitric acid, dichloromethane	5 min, 40 ℃	61	Short reaction time with moderate yield; the use of an organic solvent and the need for its separation and recovery should be considered in the overall process evaluation.	[36]
17	Isopropyl nitrate, sulfuric acid, tetrabutylammonium hydrogen sulfate, dichloromethane	0–35 ℃	36	Use of several reagents and an organic solvent; the lower yield and greater reaction complexity are additional process considerations.	[37]
18	20% nitric acid	-	-	Quantitative data suitable for direct comparison in previous nitration studies, particularly regarding 5-NSA yield, are limited, and the product yield was not explicitly reported in some of the available sources.	[38, 39]
19	Nitric acid, acetic acid	Continuous process in a confined impinging jet reactor (CIJR); SA–AcOH feed at 343 K; 69% HNO_3; molar SA:HNO_3 ratio of 5:1; reaction time <1 min	75–88	The Confined Impinging Jet Reactor (CIJR) offers rapid mixing, short reaction times, continuous operation, and scalability through numbering-up, while reducing the risk of clogging; however, isomer separation, AcOH recovery, and HNO_3 recycling remain challenges.	[40]
20	Nitric acid, sulfuric acid, acetone	Umbrella-shaped microchannel reactor; SA:acetone mass ratio of 1:5; molar SA:HNO_3:H_2SO_4 ratio of 0.5:4:1; 60 °C; residence time 4 min	85.9	Continuous microchannel operation with short residence time and substantially reduced H_2SO_4 consumption and reaction time compared with conventional batch processes. After recrystallization with 75% aqueous ethanol, 97.2% LC-based purity was achieved. The process offers improved process control, but acetone and ethanol use and the additional purification step remain relevant considerations.	[41]
21	Nitric acid, acetic acid	Continuous tubular microchannel reactor made of SS316 stainless steel; reaction time <7 min; optimized temperature for complete conversion	-	A continuous and rapid process achieving complete conversion within less than 7 min and exclusive formation of mononitrated products and enhanced selectivity toward 5-NSA. Stable operation was maintained for 2 h, while AcOH reduction caused 5-NSA precipitation and temperature increases promoted by-product formation. Solvent ratio and temperature control were therefore critical for maintaining selectivity and preventing channel clogging.	[42]
22	85% nitric acid	−5 °C	49	Direct, catalyst-free nitration, an overall yield of 93% for the isolated products.	This work

The ^{13}C NMR spectrum of 5-nitrosalicylic acid exhibited seven distinct signals corresponding to the seven carbon atoms of the molecule. The signal at δ 170.0 ppm was assigned to the carbonyl carbon of the carboxylic acid group (-COOH), while the resonance at δ 165.8 ppm was attributed to the aromatic carbon bonded to the hydroxyl group. The signal at δ 139.1 ppm was assigned to the aromatic carbon bearing the nitro group (-NO_2). 126.5, 118.4, and 113.7 ppm. These signals were assigned to the proton-bearing aromatic carbons (C-6, C-4, and C-3) and the quaternary aromatic carbon attached to the carboxyl group (C-1). The resonance at δ 129.9 ppm can be attributed to C-6, which bears a proton and is adjacent to the carboxylic acid group, while the signal at δ 126.5 ppm is assigned to C-4, also bearing an aromatic proton.

The resonance at δ 118.4 ppm corresponds to C-3, which bears a proton and is influenced by the neighboring hydroxyl and nitro substituents. Overall, the ^{13}C NMR data are consistent with the proposed structure of 5-nitrosalicylic acid [40].

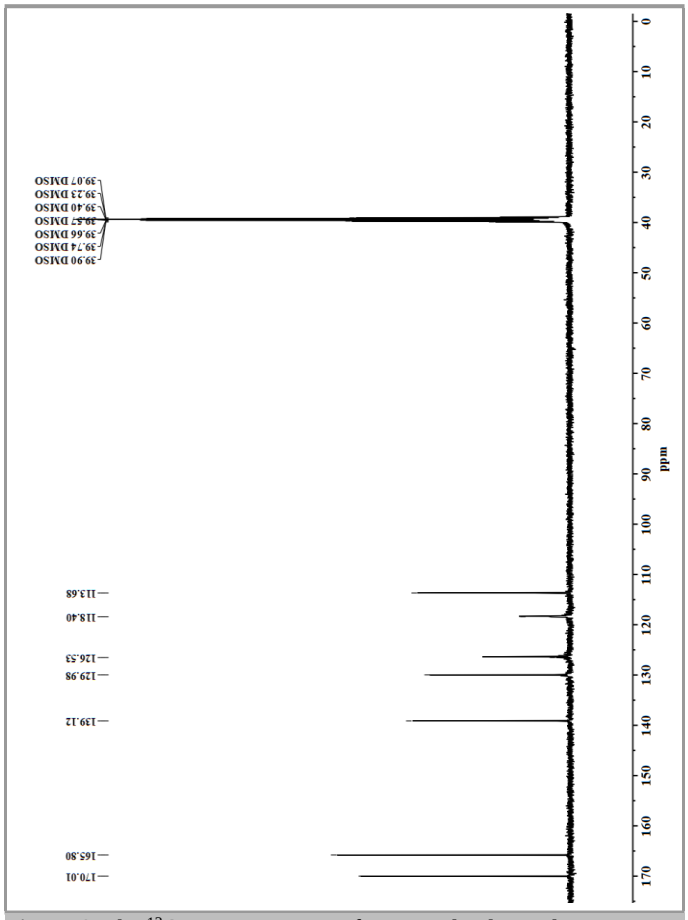


Figure 6. The ^{13}C-NMR spectrum of 5-nitrosalicylic acid in DMSO-d_6.

The DSC thermogram of 5-nitrosalicylic acid shows a distinct endothermic peak at 235 °C, corresponding to the melting point of the compound. An additional endothermic peak is observed at 303 °C, which is attributed to thermal decomposition.

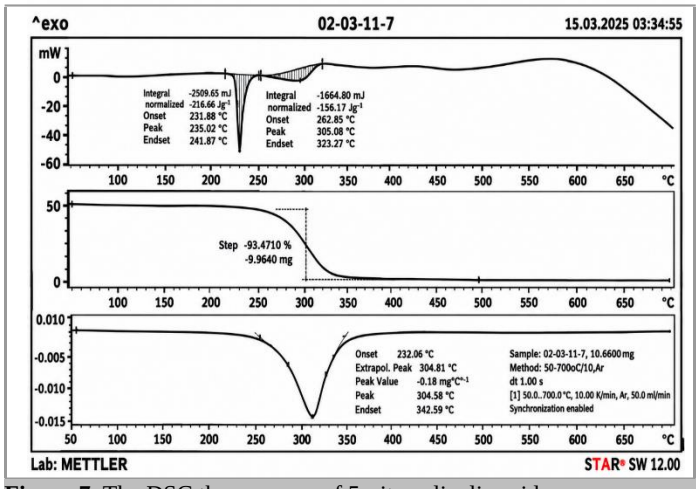


Figure 7. The DSC thermogram of 5-nitrosalicylic acid.

On the basis of results obtained from the HPLC analysis, the purity level of the resulting 5-nitrosalicylic acid is 99.9% (Figure 8).

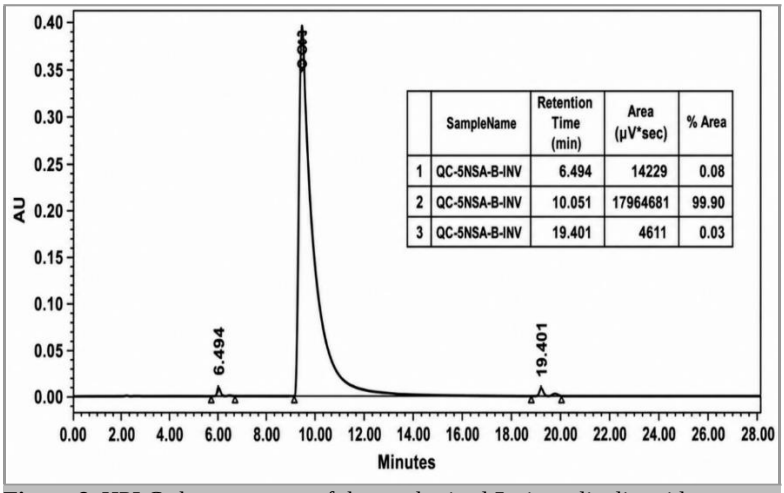


Figure 8. HPLC chromatogram of the synthesized 5-nitrosalicylic acid.

The HPLC analytical conditions for 5-nitrosalicylic acid are summarized in Table 7.

Table 7. High performance liquid chromatography (HPLC) conditions for 5-nitrosalicylic acid.

Condition	Parameter
Mode	Liquid chromatography
Detector	UV-220 nm
Column	L-1 (4.6 mm×25 cm×5µm packing)
Flow Rate	1 ml/min
Injection Volume	20 µl
Column Temperature	Room Temperature
Mobile Phase A	Dissolve 2.2 g phosphoric acid and 1.3 g perchloric acid in 1000 mL of water.
Mobile Phase B	Dissolve 1.8 g phosphoric acid and 1.1 g perchloric acid in 1000 mL Acetonitrile.
Gradient Elution	0-7 min: 100% A, 0% B 7-25 min: 100% to 40% A, 0% to 60% B 25-60 min: 40% A, 60% B

4.Conclusion

This study established a catalyst free approach for the direct nitration of salicylic acid to 5-nitrosalicylic acid, a key precursor of mesalazine. Concentrated nitric acid was employed simultaneously as the nitrating agent and reaction medium, eliminating the need for an additional catalyst or solvent. Response surface methodology (RMS) enabled the evaluation of the effects and interactions of the process variables, with the developed quadratic models showing high coefficients of determination ($R^2 > 0.99$). The RSM model-predicted optimum condition within the investigated experimental

domain was identified as −4.90 °C, 80.60% HNO_3, 188.41 min, and an SA:HNO_3 molar ratio of 1:10. The validation experiment performed under these conditions afforded 48.9 ± 0.3% of 5-nitrosalicylic acid with a total isolated yield of 92.7 ± 0.4%, demonstrating good agreement between predicted and experimental response. The optimized procedure was further validated at an increased reaction scale. Careful temperature control was essential for managing the exothermic effects associated with nitric acid dilution and the onset of nitration. Overall, the proposed method provides a simplified catalyst free nitration system based on concentrated nitric acid, with potential for further scale-up and process development.

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