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ORIGINAL RESEARCH ARTICLE

Flow injection spectrophotometry: A new approach for dapsone analysis in pharmaceutical products by coupling reaction with salbutamol sulfate

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ABSTRACT

This research developed and evaluated both batch and flow injection (FIA) spectrophotometric methods for the determination of dapsone (DAP) in pure substances and Pharmaceutical dosage forms. The proposed techniques were based on a reacting of salbutamol sulphate (SAL) with diazotized dapsone in an alkaline medium. This reaction, which occurs at 5 °C, forms a yellow dye with a maximum absorbance at 462 nm. The chemical and physical parameters of both the batch and flow injection (FIA) methods were optimized at 5 °C to maximize sensitivity and repeatability. Under these optimal conditions, Beer's law showed linearity across dapsone (DAP) concentration ranges of 3–50 µg/mL and 1–150 µg/mL. The corresponding correlation coefficients were 0.999 and 0.997, and detection limits were 0.23 µg/mL and 0.05 µg/mL for batch and flow injection FIA methods, respectively. Flow injection method (FIA) provides an economical, rapid, simple, accurate, and high-throughput approach, It is characterized by reduced consumption of samples and reagents, which minimizes waste generation. The diazotization coupling reaction with salbutamol sulfate (SAL) was selected as a safe and effective reagent for this process. This study aimed to develop a rapid and straightforward spectrophotometric method for the efficient quantitative determination of dapsone (DAP) in pharmaceutical formulations.

Keywords: dapsone (DAP); salbutamol sulphate (SAL); diazotization reaction; flow injection (FIA)

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1. Introduction

DAP, chemically designated as 4,4'-diaminodiphenylsulfone ($C_{12}H_{12}N_2O_2S$), has a (M.wt 248.31 g/mol). It presents as a white, odorless, crystalline powder with aqueous solubility. DAP is a prescription medication available in various tablet dosages for oral administration[1]. It is an aniline derivative classified within the synthetic sulfone group[2]. In 1963, the Food and Drug Administration (FDA) approved, it functions as an antibiotic and is employed in the treatment of bacterial infections in both humans and animals[3]. It has demonstrated efficacy in the treatment of bullous pemphigoid with a favorable safety profile characterized by the absence of significant side effects[4], dermatitis herpetiformis[5], pustular psoriasis[6] and leprosy[7]. Multiple analytical methods have been documented for the determination of DAP, including chromatography[8-10], chemiluminescence[11], spectrophotometry[12-14], flow injection analysis[15], methods based on charge-transfer complex formation[16] and electrochemical techniques[17]. SAL, chemically designated as "RS-1-(4-hydroxy-3-hydroxymethylphenyl)-2-(tert-

butylamino" ethanol sulfate[18], is a (β_2-adrenergic) receptor) commonly utilize as a bronchodilator in the treatment of asthma and seasonal allergies[19]. SAL has applications in sports, where it is sometimes used as a performance-enhancing drug[20]. Furthermore, it is employed as a labor inducer, in the prevention of previous labor, and as a decongestant[21]. Great doses of SAL may exhibit a lipolytic influence, and remains of this compound, primarily found in liver and meat, have the potential to be toxic to humans[22]. SAL is a recognized substance in the International, British, and United States Pharmacopoeias[23]. Various techniques have been reported for the Estimation of SAL in both pure and pharmaceutical preparations. These include flow injection spectrophotometry[24,25], voltammetry[26,27], spectrophotometry[28], capillary electrophoresis[29], fluorimetry[30], HPLC[31,32], and (GC-MS)[33]. FIA is an automated technique where a sample is introduced into a moving carrier stream. This stream then combines with reagents before the mixture is analyzed by a detector[34–38]. Flow Injection Analysis (FIA) is a cost-effective and environmentally conscious method that aligns with green chemistry principles. It requires little sample volumes (lower than 200 µL) and minimal reagent use (0.5 mL per cycle). Additionally, it offers a great sampling rate of 30 to 120 samples per hour, alongside a short analysis time and minimal waste production[39,40]. This study presents environmentally friendly batch and FIA techniques for the Estimation of DAP, employing SAL as a green and safe reagent. The FIA method, furthermore, minimizes waste generation through the use of small sample and reagent volumes. The two methods were successfully employed for the analysis of DAP in both pure and formulated forms.

2. Experimental

2.1. Instruments and FIA manifolds

A single-beam UV-Vis spectrophotometer (Shimadzu 295) equipped with a 1 cm quartz flow cell was interfaced with FIA (**Figure 1**) for absorbance measurements. FIA comprised a peristaltic pump (Ismatec, Switzerland) linked to a 6-port injection valve (Rheodyne, USA) for DAP injection. Reagent and base solutions were delivered via flexible vinyl tubing (0.5 mm i.d.). Mixing of sample, reagent, and base solutions occurred within a reaction coil constructed from Teflon tubing (0.5 mm i.d.).

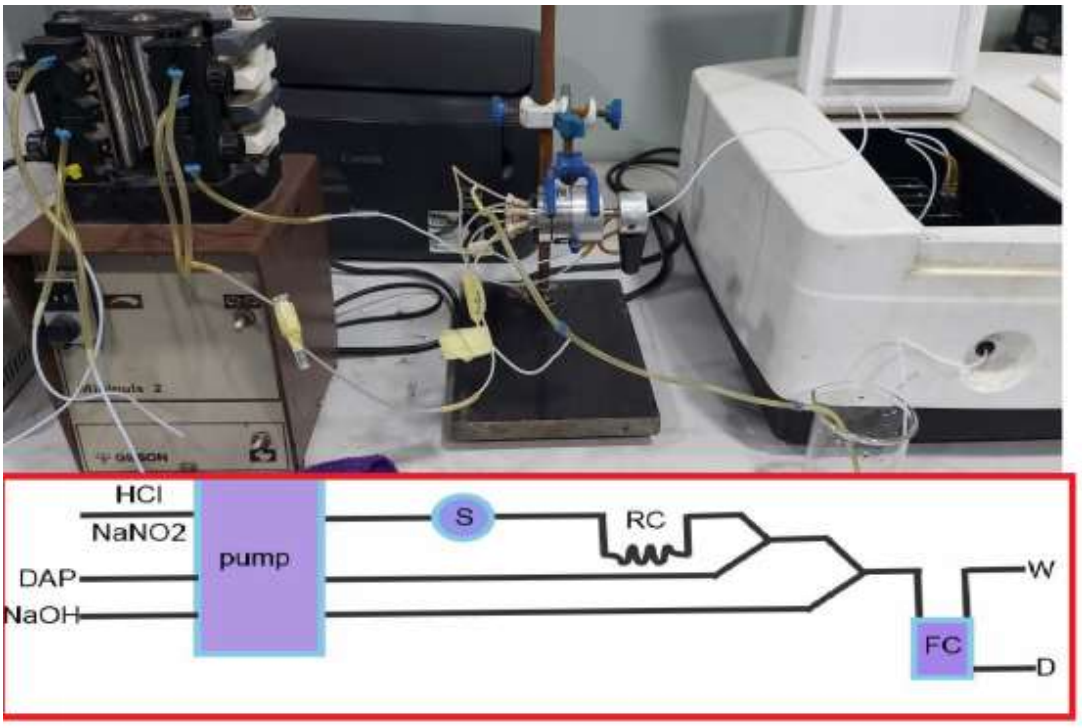


Figure 1. The FIA system's manifold was comprised of a reaction coil (R.C.), an injection port (µL), a peristaltic pump (P), a flow cell (FC), and a waste outlet (W).

2.2. Material and reagents

Pure DAP and SAL drug samples were obtained from the State Company for Pharmaceutical Industries and Medical Applications (SDI), in Samarra, Iraq. Standard stock solutions of DAP and SAL (1000 µg/mL) to prepare the solutions, 0.1 g of each pure drug was dissolved in 100 mL of distilled water. Working solutions were subsequently intended through serial dilutions of the stock solutions with distilled water. All chemicals were obtained from Merck and were of analytical grade. For the batch method, the following reagents were employed: 1% w/v sodium nitrite ($NaNO_2$), 1:1 hydrochloric acid (HCl), 10% w/v urea, and 25% w/v sodium hydroxide (NaOH). FIA method utilized the following solutions: 1 M DAP, 4 x 10^{-3} M SAL, 0.7 M HCl, 1.3 x 10^{-3} M $NaNO_2$, and 1 M NaOH. All solutions were prepared using distilled water in 100 mL volumetric flasks.

2.3. Analysis of DAP in tablets

Ten tablets of commercial dapsone (DAP) from both USA (100 mg) and India (200 mg) were individually weighed and ground into fine powders. A mass of each powder equivalent to the average tablet weight was then dissolved in 10 mL of ethanol. A small volume of distilled water was subsequently added to each solution, followed by filtration to remove any particulate matter. After filtration, each solution was moved to a 100 mL volumetric flask and topped up to the final volume using distilled water. To create the working solutions, the stock solutions were progressively diluted using distilled water.

Batch procedure

A series of DAP standard solutions, spanning a concentration range of (3–50 µg/mL), were prepared in 20 mL volumetric flasks using a 1000 µg/mL DAP stock solution. To each flask, 0.75 mL of 1:1 HCl and 0.5 mL of 1% w/v $NaNO_2$ were added. The flasks were then placed in an ice bath 5 °C for 10 minutes to facilitate diazonium salt formation. Subsequently, 0.5 mL of 10% w/v urea was added, and the mixtures were thoroughly shaken and allowed to stand for several minutes. Following this, 1 mL of 1000 µg/mL SAL solution and 1 mL of 25% w/v NaOH were added. The solutions were then diluted to the 20 mL mark with distilled water, mixed thoroughly, and maintained at 5 °C (**Figure 2**). The absorbance of each final solution was measured at λmax 462 nm against a reagent blank[41].

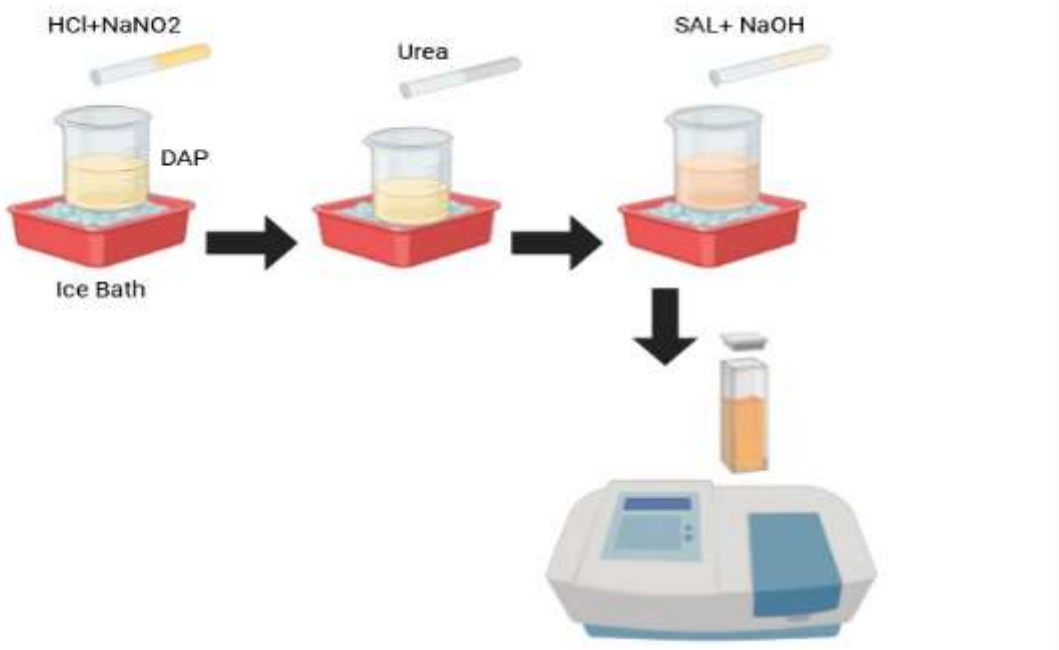


Figure 2. Diagram of diazotization reaction batch method.

FIA procedure

For FIA, 100 µL of DAP solution, within a concentration range (1–150 µg/mL) (prepared from the stock solution), was injected via syringe through the injection valve into the first channel. The second channel delivered a mixture of 0.7 M HCl and 1.3 x 10^{-3} M $NaNO_2$, the reaction was conducted in an ice bath at 5°C. This mixture was combined with the DAP sample using a T-connector to facilitate the formation of the diazonium salt. A third channel conveyed 1 M NaOH, which was then mixed with the reaction stream within a 100 cm reaction coil. SAL at a concentration of 4 x 10^{-3} M was presented into the carrier stream resulting from combination of a three channels. A peristaltic pump maintained a total flow rate of 3 mL/min for each channel. The absorbance of the yellow chromophore that formed was measured at a maximum wavelength 462 nm[42].

3. Results and discussion

Utilizing a pharmaceutical compound as a chromogenic reagent is a prioritized approach in diazotization-coupling reactions. This method offers a safer, more economical, and more sustainable alternative, as it is also environmentally friendly. Traditional amine compounds, many of which are toxic, specially those containing electron-withdrawing groups such as nitro, cyano, and halo groups, are considered less effective in this regard. In this study, a yellow dye was investigated, which was formed by the diazotization-coupling of DAP with SAL. Analysis was performed using both batch and FIA methods and was investigated to develop a more accurate and sensitive method for the detection of DAP. Chemical and physical factors were studied, including reagent concentrations, flow rate, reaction coil length, and sample volume.

3.1. Preliminary experiments of batch method

Under optimized reaction conditions, that the resulting yellow chromophore exhibited ultimate value of absorption was measured at 462 nm versus reagent blank (**Figure 3**).

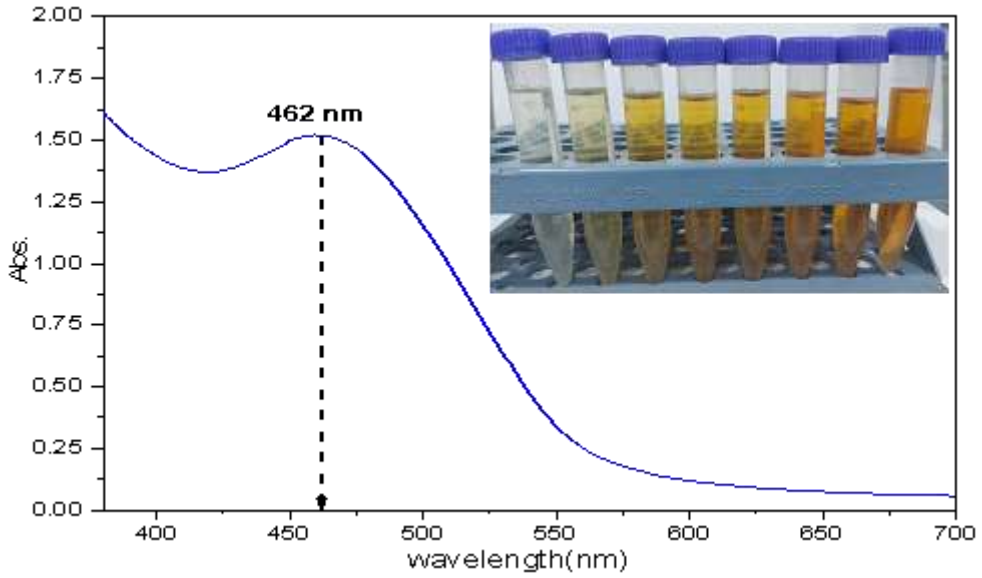
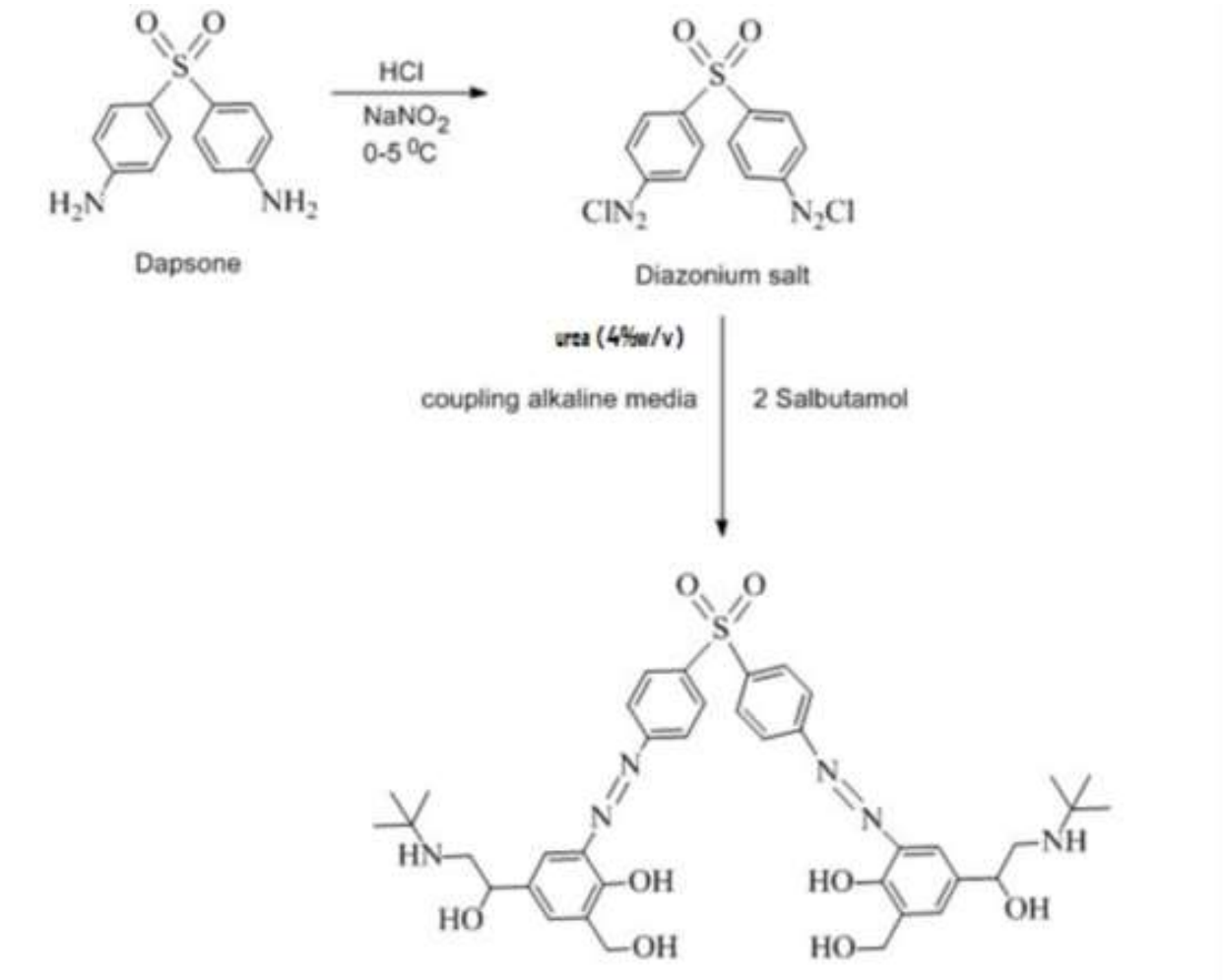


Figure 3. Absorption spectra of the yellow dye formed by reacting of 50 µg/mL DAP with SAL against reagent blank.

3.2. Optimization of azo dye synthesis from DAP and SAL

In a 20 mL graduated flask, 1 mL of Dap 1000 µg/mL was combined with varying volumes of HCl (1:1 v/v, 0.25-2 mL) and $NaNO_2$ (1% w/v, 0.25-2 mL). The reaction was conducted in an ice bath at 5°C, the highest absorbance was obtained with 0.75 mL and 0.5 mL from hydrochloric acid and sodium nitrate,

respectively. After a 10-minute reaction period, sufficient for complete diazonium salt formation (**Figure 4A-B-C**), 0.5 mL of urea (4% w/v) was append with shaking (**Figure 4D**) (it acts as an auxiliary reagent that increases the stability of the diazonium salt). Subsequently, 1 mL of SAL (1000 µg/mL) was added. The optimal volume of NaOH (25% w/v) determined by testing volumes ranging of 0.5 to 2 mL.The results indicated 1 mL of NaOH provided the highest absorbance and azo dye stability (**Figure 4E**). The reaction mixture was diluted to 20 mL with distilled water, and the resulting azo dye was monitored spectrophotometrically at λmax 462 nm. Based on the proposed mechanism is depicted in **(Scheme 1)**, the diazonium salt is prepared using an ice bath at 5°C. by reacting the amino drug DAP with nitrous acid (HCl /$NaNO_2$) in an diazotization reaction. This process converts the two amino groups in the DAP molecule to a diazonium salt, and the diazonium salt then reacts with SAL in an alkaline medium. This reaction produces a stabilized yellow dye, which can then be used for analytical purposes.



Scheme 1. A proposed mechanism DAP and SAL react to produce an azo dye.

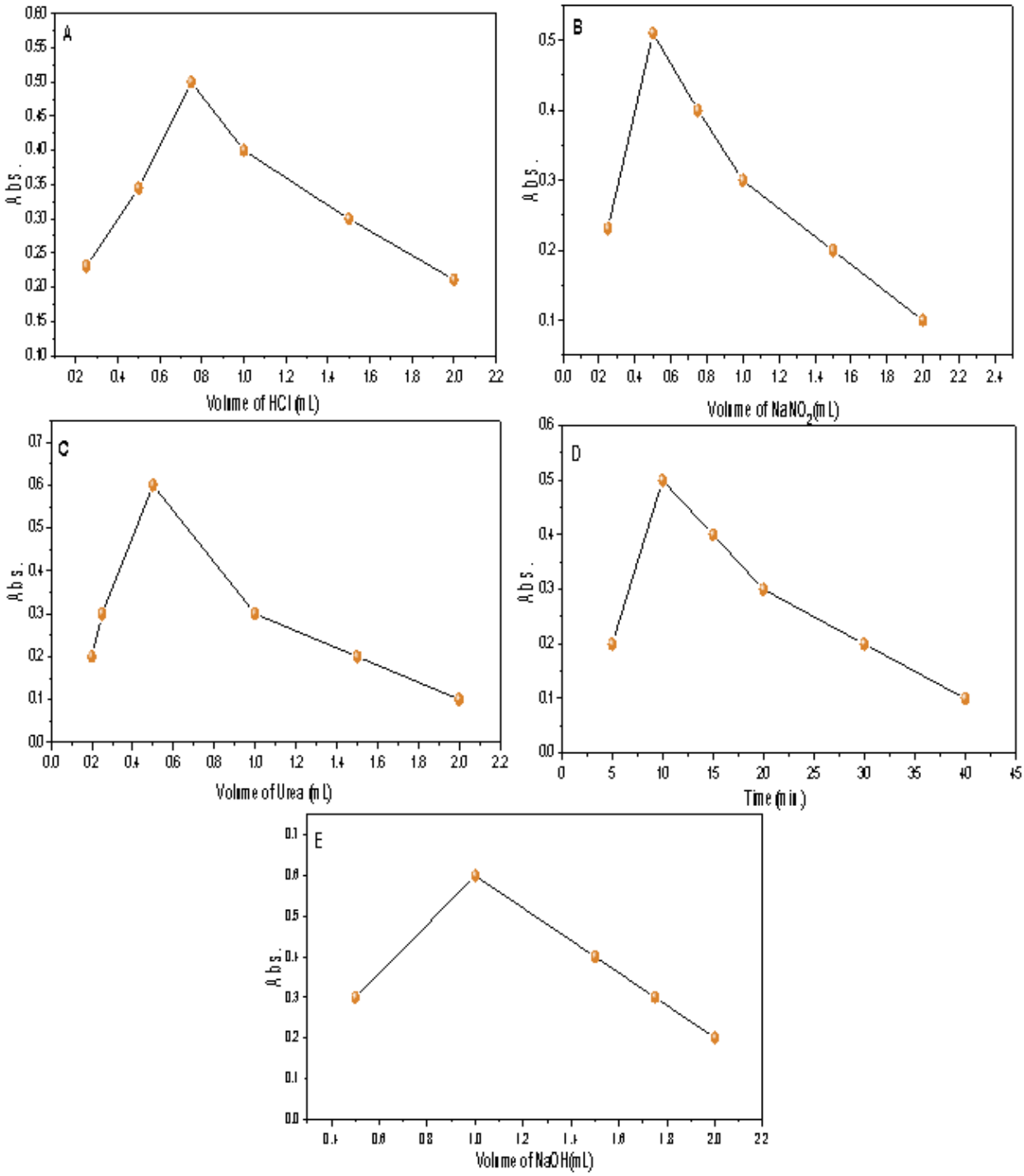


Figure 4. Investigation of Experimental Conditions for the Batch Method; Volume of Reagents and Reaction Time.

3.3. Optimization of chemical and physical variables in Flow Injection Analysis (FIA)

In an ice bath at 5 °C. The chemical variables influencing the reaction were systematically investigated Hydrochloric acid (HCl) and sodium nitrite ($NaNO_2$) concentrations were varied across the ranges of 0.2-2 M and 0.4 x 10^{-3} - 2 x 10^{-3} M, respectively. Optimal absorbance was achieved with 0.7 M HCl and 1.3 x 10^{-3} M $NaNO_2$ (**Figure 5A-B**). SAL concentration was studied within the extent of 1.5 x 10^{-3} - 6.5 x 10^{-3} M, with highest absorbance observed at 4 x 10^{-3} M. Given the established requirement of an alkaline medium for the reaction between SAL and the diazonium salt, sodium hydroxide (NaOH) concentration was optimized across a range of 0.5-4 M. A 1 M NaOH solution yielded the best results. Following chemical optimization,

the impact of physical variables was examined. Injected sample volume (1 M DAP) was varied from 1 to 150 µL (**Figure 5C**). A 100 µL injection volume provided the highest absorbance. Volumes exceeding 100 µL resulted in decreased absorbance, likely due to a higher sample-to-reagent ratio and increased dispersion. The effect of mixing coil length (25-250 cm) on the sensitivity of the colored reaction product was also investigated (**Figure 5D**). A 100 cm coil length yielded maximum absorbance, providing sufficient mixing time. Lengths greater than 100 cm led to decreased absorbance due to increased sample dilution. Consequently, 100 cm was selected as the optimal coil length. Flow rate was studied across a range of 1-5 mL/min. Maximum absorption was observed at 3 mL/min, with decreased absorption at higher flow rates. This decrease is attributed to increased sample dilution, dispersion, and reduced reagent mixing time above 3 mL/min (**Figure 5E**). Therefore, 3 mL/min was chosen as the optimal flow rate. All optimized chemical and physical parameters are summarized in **Table 1**. The maximum absorption of the resulting yellow solution was observed at λmax 462 nm.

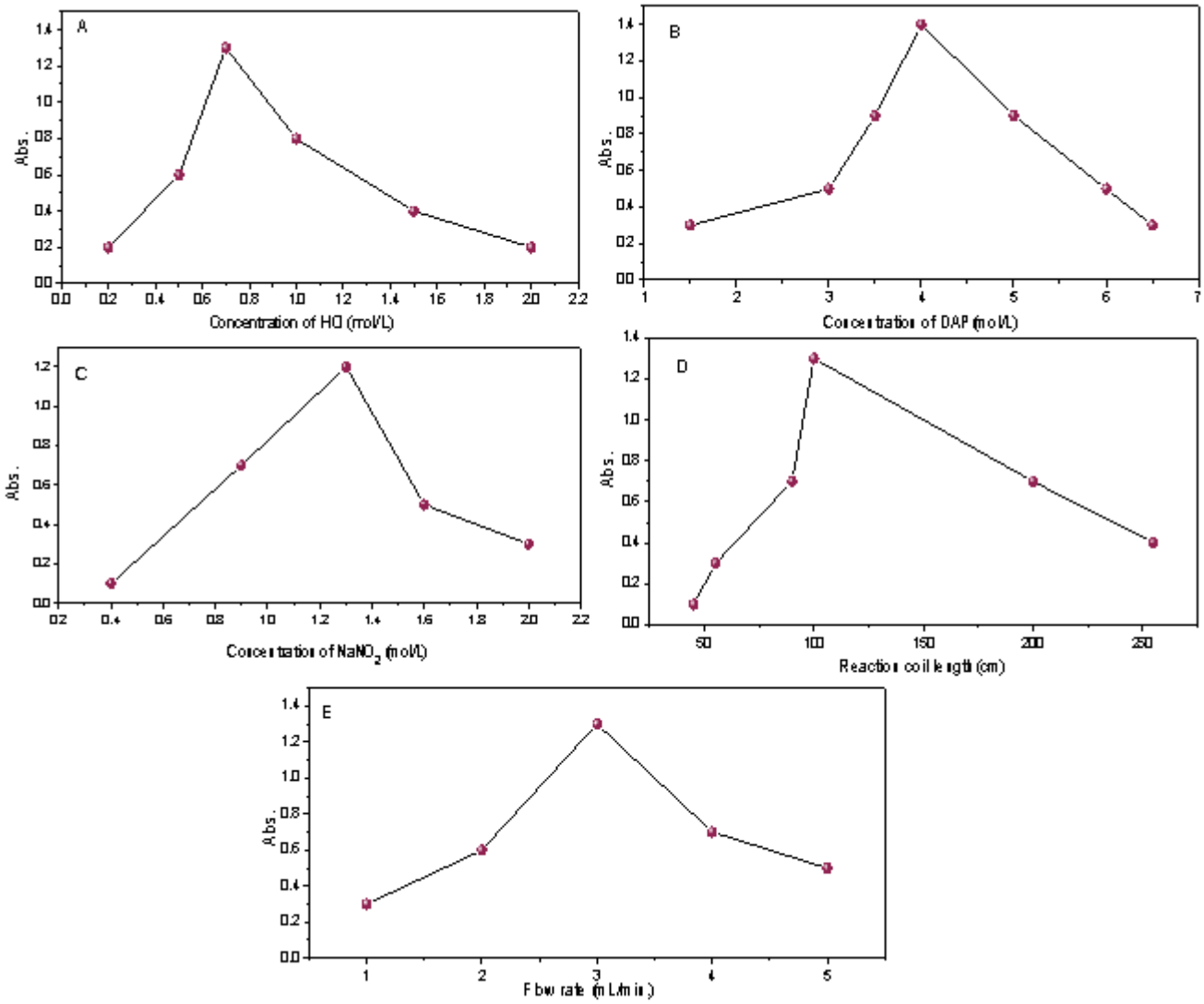


Figure 5. Effect of the concentration of (A) HCl, (B) $NaNO_2$, (C) DAP, (D) reaction coil length and (E) flow rate.

Table 1. Optimized physical and chemical parameters for DAP determination using flow injection analysis.

Variables	Ambit of study	Ideal value
Concentration of HCl (M)	0.2-2	0.7
Concentration of (1%) $NaNO_2$ (M)	0.4×10^{-3}–2×10^{-3}	1.3×10^{-3}

Variables	Ambit of study	Ideal value
Concentration of SAL (M)	1. 5×10^{-3}–6.5×10^{-3}	4×10^{-3}
NaOH (M)	0.5 – 4	1
volume of the injected sample of 1 M of DAP (µL)	1 – 150	100
Reaction coil length (cm)	25 – 250	100
Flow rate (mL/min)	1- 5	3

Table 1. (*Continued*)

3.4. Analytical performance characteristics

Calibration curves were established for both the batch and FIA methods by plotting absorbance values against corresponding DAP concentrations, following optimization of all reaction parameters (**Table 2** and **Figure 6**). **Table 2** presents a summary of the analytical data obtained from the calibration curves, including the slope (a), intercept (b), and coefficient of determination (r^2). These data demonstrate the high precision and sensitivity achieved with the proposed methods.

Table 2. Analytical and regression parameters for DAP analysis utilizing diazotization and FIA techniques.

Parameters	Diazotization	FIA
λ_{max} nm	462	462
Color	Yellow	Yellow
Linearity range (µg/mL)	(3−50)	(1-150)
Regression equation	Y=0.017x+0.018	Y=0.007x+0.067
Ɛ (L/mol.cm)	$0.1x10^5$	0.3×10^7
Correlation Coefficient (r)	0.999	0.997
Intercept (a)	0.018	0.067
Slope (b)	0.017	0.007
Sandal'sensitivity (µg/cm^2)	0.056	0.001
Limit of detection LOD (µg/mL)	0.23	0.05
Limit quantification LOQ (µg/mL)	0.64	0.16

LOD = 3.3 x SDb/b, LOQ= 10 x SDb/b, SDb = Standard deviation of blank,b= is a slope of calibration curve[43,44].

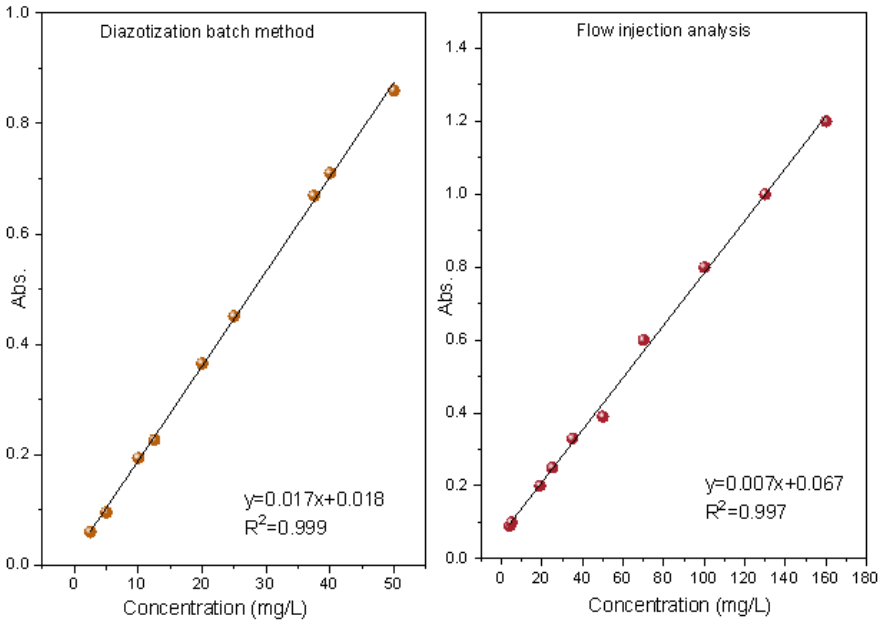


Figure 6. Calibration curves of suggested methods

3.5. Reproducibility and accuracy

The accuracy and precision of both methods were evaluated by analyzing three various DAP concentrations in replicates of at least five under optimized conditions. Accuracy was assessed by calculating percent recovery, while precision was estimated by calculating the relative standard deviation (RSD). The results, presented in **Table 3**, demonstrate the acceptable accuracy of the proposed methods.

3.6. Applications within the pharmaceutical field

The two suggested techniques were applied to the quantitative estimation of DAP in pharmaceutical formulations. Two types of DAP tablets, containing 100 mg and 200 mg of DAP per tablet and sourced from the USA and India, were analyzed. The results (**Table 4**) demonstrate good recovery and the absence of significant interferences.

Table 3. Assessing the Accuracy and Precision of the suggested Methods for Pure DAP.

Method	Amount of drugs (µg/ml)		Relative Error %	Recovery %	Average Recovery%	RSD% (n=5)
	CT	CF				
Diazotization	5	4.97	-0.60	99.4	99.98	0.60
	10	9.99	-0.10	99.9		0.30
	15	15.10	0.66	100.66		0.19
FIA	5	4.87	-2.6	97.40	99.34	0.07
	10	9.85	-1.50	98.50		0.04
	15	15.32	2.13	102.13		0.02

The concentration of DAP is represented by CT and CF. The results also include relative error (E%), recovery (Rec%), and relative standard deviation (RSD)

Table 4. Quantification of DAP in commercial tablet preparations.

Tablet of DAP	Method	Amount of drugs (µg/ml)		Relative Error %	Recovery %	Average Recovery%	RSD% (n=5)
		Taken	Found				
Tablet USA 100 mg	Diazotization	10	9.93	-0.68	99.31	99.86	1.17
		20	20.02	0.13	100.13		0.66
		30	30.07	0.24	100.14		0.30
Tablets Indian 200mg		5	4.91	-1.68	98.32	99.81	1.64
		20	19.96	-0.16	99.84		0.51
		30	30.38	1.29	101.29		0.51
Tablet USA 100 mg	FIA	40	39.75	-0.63	99.37	98.87	0.92
		80	78.81	-1.49	98.51		0.34
		100	98.74	-1.26	98.74		0.27
Tablets Indian 200mg		10	10.18	1.80	101.80	99.94	0.95
		30	29.73	-0.90	99.10		0.5
		70	69.26	-1.06	98.94		0.26

3.7. Comparison with existing spectrophotometric methods

The proposed methods for DAP determination were compared against spectrophotometric techniques already documented in the literature (**Table 5**). The data indicate that the current methods exhibit comparable or improved sensitivity compared to existing techniques. Furthermore, the limits of detection

(LOD), limits of quantification (LOQ), and linearity of the proposed methods are align with findings from other published research.

Table 5. Comparison of proposed and recent methods for DAP analysis.

Method	LOD (µg/ ml)	LOQ (µg/ ml)	Linearity (µg/ ml)	λmax (nm)	Ref.
A Spectrophotometric Method Utilizing a Diazotization Reaction	0.0260	0.0867	0.1–2.5	570	45
A Spectrophotometric Method Utilizing a Diazotization Reaction	0.0422	0.1281	12 –14	470	46
A spectrophotometric FIA method utilizing a diazotization raction	5	…..	100	485	15
HPLC	0.41	1.24	5-25	230	9
TLC	8.96 × 10–3	29.88×10^{-3}	0.5–6	289	10
HPLC	6.2 × 10–2	20.6×10^{-2}	5-65	289	10
A spectrophotometric FIA method utilizing a diazotization raction	0.023	0.075	0.1-1.8	460	14
A spectrophotometric FIA method utilizing a diazotization raction	0.05	0.16	1-150	462	This paper

4. Conclusion

The proposed batch and FIA methods have been Apply successfully to the estimation of DAP in both bulk and pharmaceutical formulations. Furthermore, FIA method utilizes SAL, a non-hazardous reagent, which reacts with the diazonium salt in an alkaline medium to form the azo dye. FIA method provided many advantages as simplicity, low cost, great precision precision, sensitivity, and rapid analysis. Finally, compared to the other methods, FIA method significantly reduces the consumption of chemicals and reagents, resulting in diminished waste generation.

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Conflict of interest

The authors declare no conflict of interest.

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