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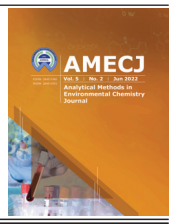

Design a continuous microfluidic flow cell for turbidimetric-flow injection technology: A new approach for routine analysis of active pharmaceutical formulations

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ABSTRACT
In this study, a new flow injection analysis (FIA) based on a microfluidic flow cell (MFC) with a sample capacity of 40 µL is described. A Tungsten lamp directs light from a typical 2100P Portable Turbidimeter apparatus into a quartz flow cell through a round sidewall aperture of 2.0 mm and emerges through the identical aperture on the opposite side of the flow cell, where a photodiode array (light detector) detects the passing light. When compared to a traditional cuvette (25 mm x 60 mm round) with the same nominal route length, this technique improves sensitivity by around 4.0. This improvement is due to the use of a short, narrow internal diameter microfluid as the flow cell, which reduces physical dispersion. The designed flow cell has been evaluated by developing a turbidimetric method for the detection of promethazine in pure form or pharmaceutical dosages. The developed method is based on forming of a yellowish ion-pair association complex due to the reaction of promethazine and sodium tetraphenylborate (STPB) in an acidic medium. At the flow optimum conditions, the calibration curve (CC) and the limit of detection (LOD) for promethazine were obtained 0.5-90 µg mL^{-1} and 0.35 µg mL^{-1}. respectively (R^2 = 0.9955). The intra-day and inter-day precisions (RSD%) of the FIA-MFC method for measuring promethazine at concentrations of 20, and 50 µg mL^{-1} were achieved (2.0 and 1.6) and (0.8 and 1.2), respectively.

1. Introduction

Promethazine, also known as (2RS)-N, N-dimethyl-(10H-phenothiazine-10-yl) propan-2-amine, is an antihistaminic having analgesic, anticholinergic, antipsychotic and sedative effects that are utilized to treat vomiting, nausea and motion sickness in children [1]. In the wide group of phenothiazine derivatives, promethazine hydrochloride is a well-known chemical. It's a crystalline powder that's white or slightly yellowish in color, extremely soluble in water, and easily soluble in methylene chloride and alcohol. Morphine sulfate, benzylpenicillin salts, hydrocortisone sodium succinate, aminophylline, alkalis, barbiturates, heparin, and various contrast solutions are incompatible with promethazine. Tachycardia, bradycardia, transient small elevations in blood pressure, and infrequent hypotension are all side effects. Extrapyramidal symptoms have been described at high doses, such as jaundice and thrombocytopenic purpura. Excessive sedation, impaired dizziness, motor function, tachycardia, extrapyramidal symptoms, disorientation, blurred

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vision, palpitations, stomach distress, headache and constipation are all adverse effects of promethazine [2]. As a result, according to the substance act [3], promethazine is classified as a dangerous drug and is not sold over the counter. The purposeful, non-therapeutic use of a drug, even once, for its favorable psychological benefits is known as prescription drug misuse. Promethazine recreational usage among college students and high school has become a severe concern, as documented in Hong Kong, Nepal, the United States, and India [4]. Antihistamine medicines like promethazine are often abused by mixing with energy drinks, candies, soft drinks, and alcoholic beverages in cocktails [5-7]. Promethazine detection has been reported using a variety of analytical approaches. Spectrophotometry [2], gas chromatography [8], HPLC [9], electrochemical sensing [10], and capillary electrophoresis [11] are some of the techniques used. All of these approaches, however, have disadvantages for routine promethazine analysis in pharmaceutical formulations samples. Specific reagents such as 12-tungstophosphoric acid (TP) [12], potassium persulfate [13] and cerium (IV) [14] are what is the colorimetric detection based on. However, spectrophotometry instrument is not ideal for field use or on-site detection, and this method is mainly applied to samples solutions. Traditional separation procedures have significant drawbacks when it comes to separating and identifying individual components in complicated mixtures. They may necessitate time-consuming sample preparation, expensive equipment, and well-trained analysts [15, 16]. The oxidation of Promethazine can be conducted in an electrochemical flow cell rather than a chemical reaction, and the amperometric signal can be utilized to detect it. Interference is likely to be occured when there are many electroactive compounds in the sample, depending on their oxidation potential [17]. Voltammetric detection is more difficult, although it can be used when the peaks do not overlap much [18]. Because of the presence of linked pharmaceutical substances or dyes, neither approach is completely suitable for determining Promethazine in a variety of pharmaceutical formulations. In recent years, a growing number of Flow Injection Analysis (FIA) techniques for the determination of Promethazine have been described [10, 19-22]. Almost all of them investigate the practicality of in-line oxidation of the analyte by an immobilized oxidant or confluent, accompanied by spectrophotometric detection of the colored radical, stabilized under very acidic conditions, while others look into the fluorescence effect. The need for acid-resistant tubing for the flow manifold system, as well as the danger of interference from colored excipients, which can vary from one commercial formulation to the next and may absorb at the wavelength chosen for the analyte, are some of the drawbacks of spectrophotometric procedures. Although spectrometry has been used to study electrostatic interactions in phenothiazines, it has also been used to investigate analyte detection procedures that involve colored intermediates or products. However, its application for routine analytical purposes is scarce, perhaps because the cells are difficult to construct or operate, or because they were not designed for FIA [23, 24]. The turbidimetric-flow injection technique is a quicker alternative to spectrophotometric methods for evaluating active pharmaceutical ingredients, as it can produce results in as little as one minute. Moreover, the turbidimetric method has the advantages of low consumption of samples and reagents, high accuracy, simple operation and quick analysis. Therefore, several turbidimetric-FIA procedures have been developed for the determination of active pharmaceutical ingredients in different matrices [25-29]. To overcome the limitations associated with the spectrophotometric-flow injection technique, an inexpensive, very simple and long-optical-path turbidimetric-flow cell is designed and evaluated. This quartz cell is resistant to high temperatures and corrosion. The flow cell is cylindrical, 50 mm in length, and features 2 mm inside and 4 mm outer diameter input and output holes. The developed cell is compatible with an exit manifold system for interfacing to tubing and it requires only a simple

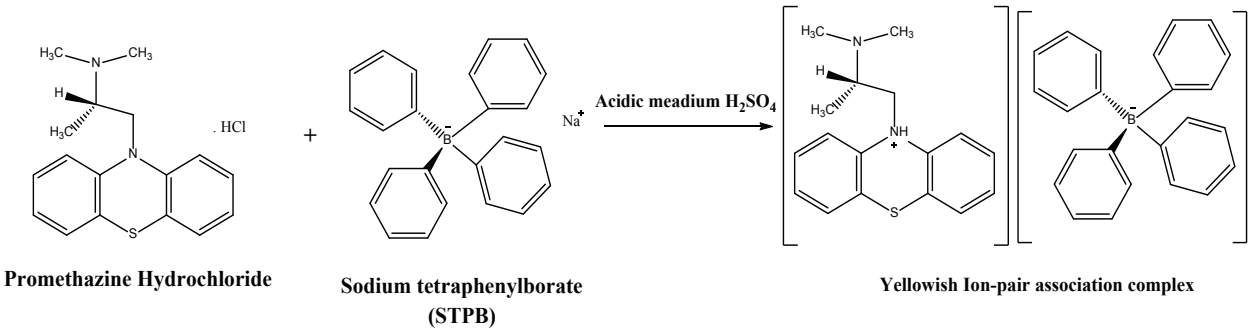

Fig. 1. The proposed mechanism of the reaction between the promethazine hydrochloride and the STPB reagent

benchtop apparatus for detecting and measuring the samples or any standard materials that pass through it. The designed flow cell was successfully used to develop and validate an analytical method for the determination of Promethazine in pure and pharmaceutical formulations to evaluate the flow cell. Also, many techniques such as LLME and SPE was used for sample preparation in human and environment.

The developed method is based on forming of a yellowish ion-pair association complex due to the reaction of promethazine and sodium tetraphenylborate (STPB) in an acidic medium, the proposed mechanism for the reaction is shown in Figure 1. The simplicity, flexibility and effectiveness of this new turbidimetric-flow cell approach opened up new avenues for investigating the current approach in analytical analysis applications, as shown here in the determination of promethazine hydrochloride. It's perfect for routine analysis of promethazine hydrochloride in pure form or dosage forms containing active substances, inert ingredients, and dyes.

2. Experiment

2.1. Reagents and solutions

All solutions and analytical grade reagents were prepared with double-distilled water. Dilution of concentrated sulfuric acid acid produced an appropriate concentration of a sulfuric acid solution (0.2 mol L^{-1}). Working solutions were prepared daily by diluting suitable aliquots of promethazine hydrochloride ($C_{17}H_{20}N_2S$, HCl, 320.9 g mol^{-1}, SDI) into double distilled water. The purity of the promethazine was determined to be 99.7 % using an official method [30]. A stock solution of Sodium tetraphenylborate (STPB), $C_{24}H_{20}BNa$, 342.22 g ml^{-1}, BDH 50 µg mL^{-1} by dissolving 4.2777g per 250 mL distilled water. Sulphuric acid, hydrochloric acid, nitric acid, acetic acid, ammonium chloride, sodium nitrate, potassium bromide and sodium chloride were purchased from Sigma Aldrich.

2.2. Preparation of standard and sample solutions

0.049 mg of promethazine was weighed and diluted in 250 mL of double-distilled water to produce the standard stock solution (200 µg mL^{-1}). After that, the stock solution was kept in an amber container for the subsequent research. Dilutions of appropriate quantities of stock solution with double-distilled water were used to create standard solution ranges. The stability of promethazine was monitored frequently using UV spectrophotometry to see if there was any decomposition during the development of the method, and the results showed that promethazine was stable for 20 days. Two distinct commercial companies of 25 mg were used in this study. The pharmaceutical preparations tested were Histazine tablets (25 mg per tablet) by United pharmaceutical Jordan and Phenergan tablets (25 mg per tablet) by GLOBAL manufacturer Sanofi United pharmaceutical UK, these tablets are available in most countries. Twenty tablets were powdered and dissolved in double-distilled water after being precisely weighed. The sample solution

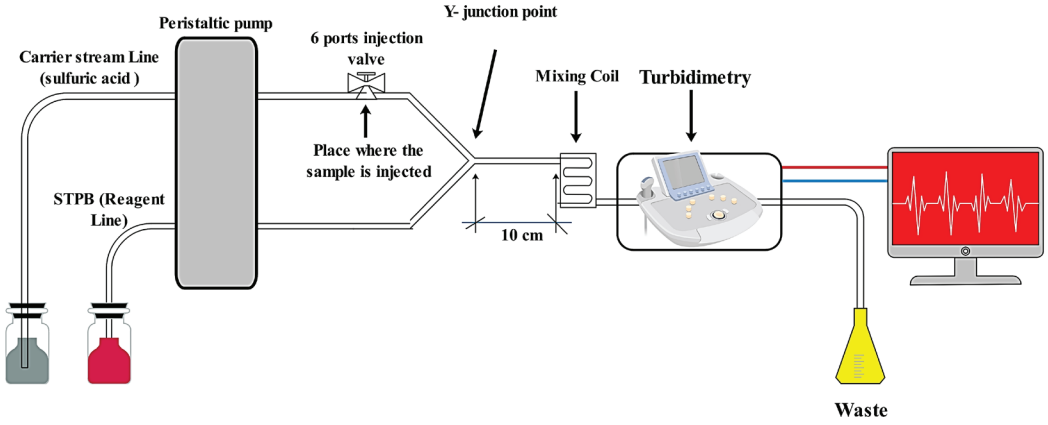

Fig. 2. The whole manifold system used to determine the promethazine hydrochloride

was then filtered after being shaken for 20 minutes. The various concentrations of the injected sample solutions were obtained by diluting the prepared stock solution appropriately. For each commercial tablet type, the method has been repeated.

2.3. Apparatus

All the turbidimetric measurements were conducted using a conventional 2100P Portable Turbidimeter (USEPA 180.1, EU), modified in the authors' laboratory and interfaced to the computer where all data and measurements are recorded and stored. The Portable turbidimeter is also interfaced with the PC controlled by software supplied by the same company (HACH). A manual rotating injector (6 ports) and an Ismatec 2 channels (ISM796, supplied with Tygon pump tubing, Switzerland) peristaltic pump were employed in the FIA experiments. All of the studies were carried out at room temperature. Figure 2 illustrates the manifold used in the promethazine hydrochloride assay. Teflon (IDEX corporation, USA) tubes were used to connect all the manifold system parts.

A flow cell was designed and used for all of the turbidimetric experiments; this quartz cell is resistant to high temperatures and corrosion. The flow cell is 50 mm long and has input and outlet ports with an interior diameter of 2 mm and an outer diameter of 4 mm. It is through this flow cell that the samples and any standards are passed for detection and measurement by the turbidimetric detector as shown in Figure 3.

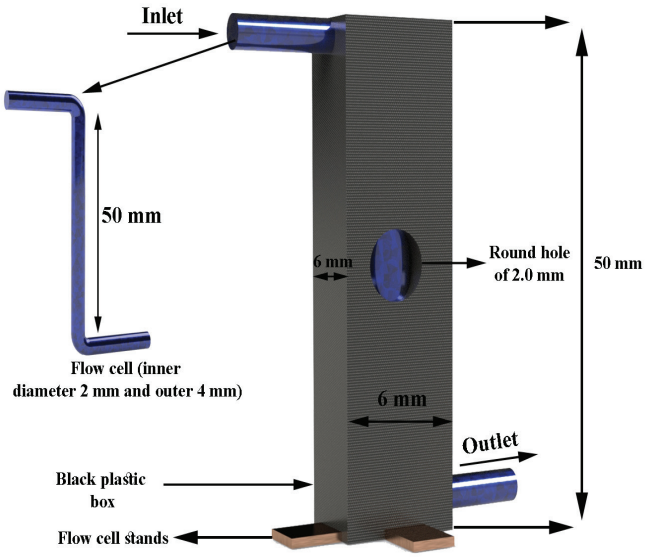

Fig. 3. The complete description of the developed flow cell

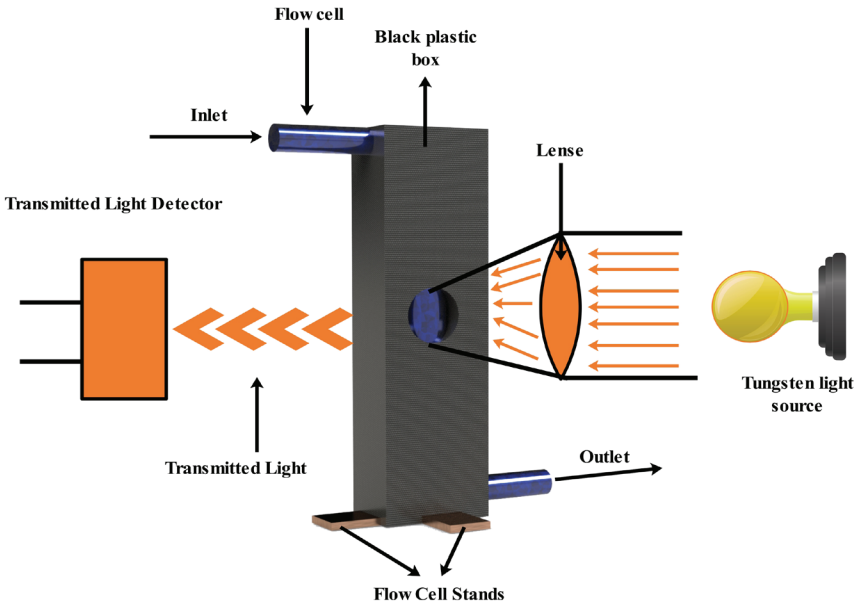

Fig. 4. The front view of the design flow cell assembly. The tungsten light source is mounted directly on the round hole of the flow cell and the radiation of the light source is concentrated on the flow cell hole using a lens. In contrast, the flow cell sits on the floor stage mounted vertically on the platform using three metal stands. The transmitted light detector is introduced on the opposite side of the flow cell.

As illustrated in Figure 4, the flow cell is inserted vertically into a black plastic box with a diameter of (50 mm (L) 6 mm (W) 6 mm (thickness)). This plastic box can occupy the entire flow cell. The sidewall of the black box contains a round hole of 2.0 mm which is suitable for the source of light that radiates from the turbidity device, the light source was fully mounted and constructed. While the opposites side of the box is supplied by the light detector (photodiode array) that is used to measure the passing light through the flow cell. A silicone rubber tubing sleeve with the necessary inner diameter was used to connect the constructed flow cell to the FIA manifold system, and the entire cell assembly was placed on a robust metal base plate and encased in a black plastic box.

2.4. General Procedure

Figure 2 illustrated the manifold flow apparatus that was used to determine promethazine in pharmaceutical preparations. This system consisted of two lines: the carrier streamline (20 Mmol L^{-1}, sulfuric acid) and the reagent line solutions (STPB 30 µg mL^{-1}). Both lines were accelerated using a two-channel peristaltic at flow rates of 2.0 ml min^{-1} for the acid line and reagent line, respectively. The injection coil of the injection valve is first loaded with (100 µL) of aliquot volume from a sample solution that contains promethazine. The injection valve's position is then adjusted from loading to the injection mode. The sample solution is injected into the acidic streamline and merged with the reagent at the Y junction point (one of the manifold system parts, made from Methyl methacrylate) to generate the yellow complex product, which is then delivered by the stream to the detector system for analysis. The formed complex's turbidity measurements will be recorded and stored continually by PC indicated by (peak). The instrument is calibrated to be set to a signal of zero for the blank signal each time it is used since the blank (STPB) has a signal that is almost zero. The calibration graph used to calculate promethazine is then shown with the corresponding peaks. The created product might adsorb on the

wall of the quartz flow cell since it is turbid, which will result in inaccurate findings being obtained. To address this issue, a washing buffer solution containing 7 g ammonium chloride, 57 mL 25 % (m/m) ammonia, and 40 g EDTA (disodium salt) was made by dissolving the various ingredients in 500 mL distilled water and diluting to 1.0 L [31]. After each sample injection, 10.0 mL of washing buffer solution is utilized to ensure that any adsorption particles on the flow cell's surface are removed.

3. Results and Discussion

The turbidimetric and spectrophotometric responses were measured concurrently in all of the experiments. The operating conditions were extensively studied and optimized, including the STBP concentrations, flow rate, injected-sample volume, carrier stream, mixing coil and analyte concentration. Studies were carried out by changing one variable at a time while keeping the others unchanged. When measuring promethazine hydrochloride in Histazine and Phenergan tablets using analytical curves or the method of standard additions, the results obtained were sufficient and reliable enough to propose the methodology as an alternative to the British Pharmacopoeia's official methods with confidence [30].

3.1. Investigation of FIA parameters

The quantitative determination of compounds using a flow system necessitates the optimization of several physical and chemical parameters, including the type of carrier stream, reagent concentration, injected-sample volume, flow rate, and mixing coil. All of these trials were optimized under flow conditions. First, a series of STPB concentrations ranging from 0.5 to 50 µg mL^{-1} were generated while other parameters such as sample volume (50 µL), flow rate (1.0 mL min^{-1}), and open valve mode were held constant. The injection valve in use has two settings: closed and open. Closed valve mode implies rotating the valve from the injection to the loaded position after some time, whereas open valve mode means leaving the valve in the injection position and allowing the carrier stream to pass through it to transport the sample to the detector. The goal of these modes is to figure out how long it takes to transfer all of the sample segments using the carrier stream. The results showed a considerable rise in the response profile up to 30.0 µg mL^{-1}, beyond which there was no increase in the detector signal. As a result, the optimal concentration of 30.0 µg mL^{-1} was used in Figure 5.

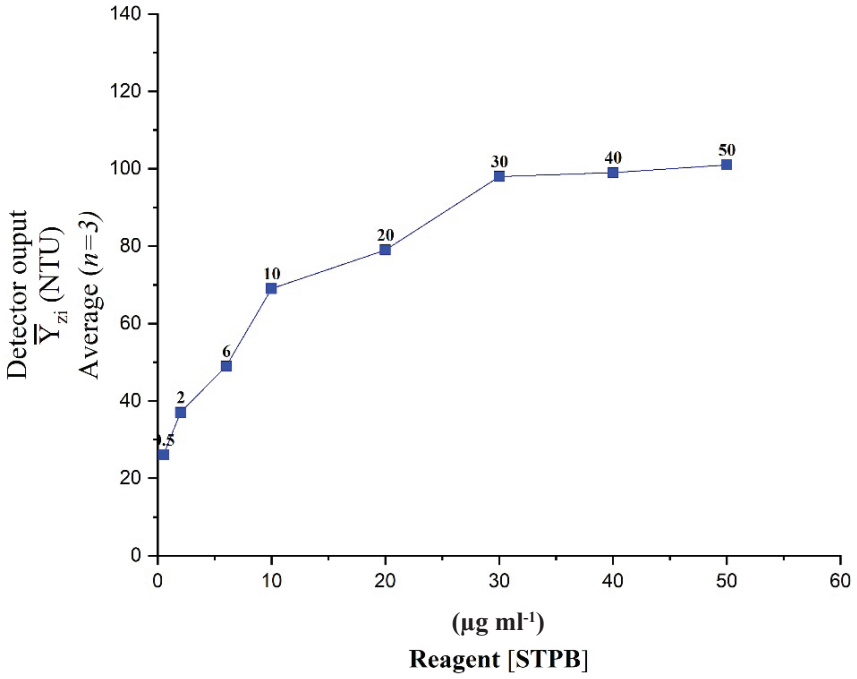

Fig.5. The effect of STPB Conc. [µg ml^{-1}] on the determination of promethazine using experimental conditions: 100 µL of promethazine (50 µg mL^{-1}), flow rate 1.0 mL min^{-1} and open valve mode

In most flow systems, double-distilled water serves as the carrier stream; however,using other solutions instead of water may enhance the detector signal; as a consequence, a series of 25.0 mmol L^{-1} solutions (H_2SO_4, HNO_3, HCl, CH_3COOH, NH_4Cl, $NaNO_2$, KBr, NaCl, KNO_3) were used as a carrier stream in place of water. These solutions were chosen at random, and some of them have an anion and cation activity, while the remainder is acid and basic medium. The major reason to use these alternatives rather than water is that the interaction between the sample solution and the organic reagent occurs in an aqueous medium (distilled water), resulting in the formation of a new colorful product. As a result, it was necessary to determine whether the new product is steady in the aqueous solution or if it will be dissolvable and some of it will solubilize in that solution, as we are always searching for a product with chemical stability in the flow system. The highest detector signals were obtained using diluted sulfuric acid as the carrier stream across all of these solutions. This result can indeed be attributed to the fact that the formed product, which is developed as a result of the reaction between both the organic reagent (STPB) and the promethazine, is more stable in sulfuric acid solution than in distilled water, and this is connected to the fact that sulfuric acid inhibits promethazine precipitation during promethazine dissociation. As a result, sulfuric acid was chosen as the carrier stream (Fig. 6).

After agreeing to employ sulfuric acid instead of water as the carrier stream, the concentration of it had to be adjusted, therefore varied concentrations ranging from 10 to 50 mmol L^{-1} were prepared and then used under previous flow conditions. Because the results revealed that 20 mmol L^{-1} sulfuric acid was adequate to achieve a strong signal, 20 mmol L^{-1} was selected in subsequent experiments (Fig.7). Sulfuric acid 20 mmol L^{-1}, promethazine 30 µg mL^{-1}, 1.0 mL min^{-1} flow rates are the flow conditions that are applied to conduct the injected-sample volume experiment; therefore, the influence of the injected-sample volume was studied, and a series of sample volumes ranging from 30-150 µL were injected into the manifold system that utilizes a 6-port injection valve. The acquired data revealed that the response profile increased up to 100 µL, but that applying more than that volume resulted in a lowering in the response profile owing to precipitation of the generated product in the flow cell and decreased incident light attenuation. As a result, as shown in

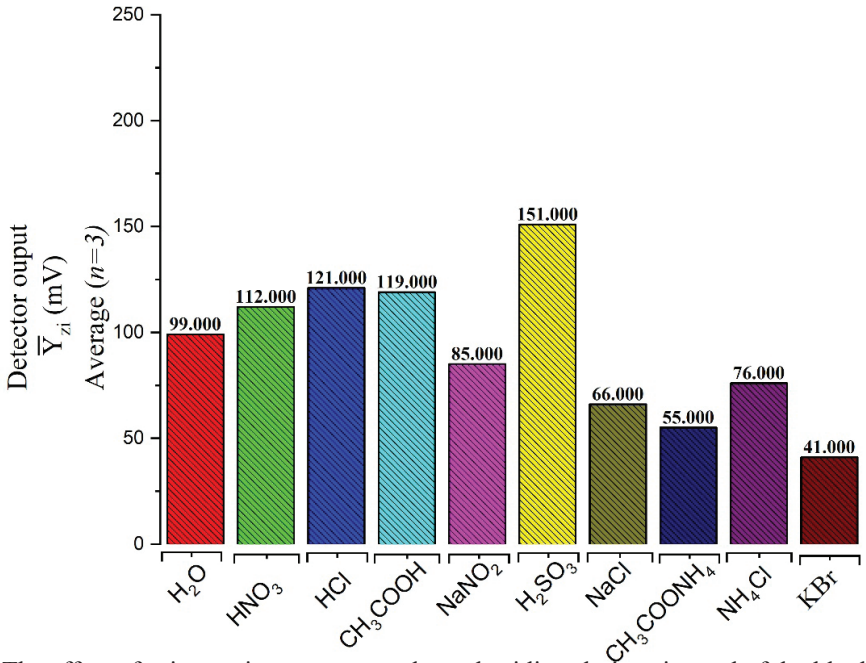

Fig. 6. The effect of using various aqueous salts and acidic solutions instead of double distilled water as a carrier stream on the detector signal under using the same initial flow conditions

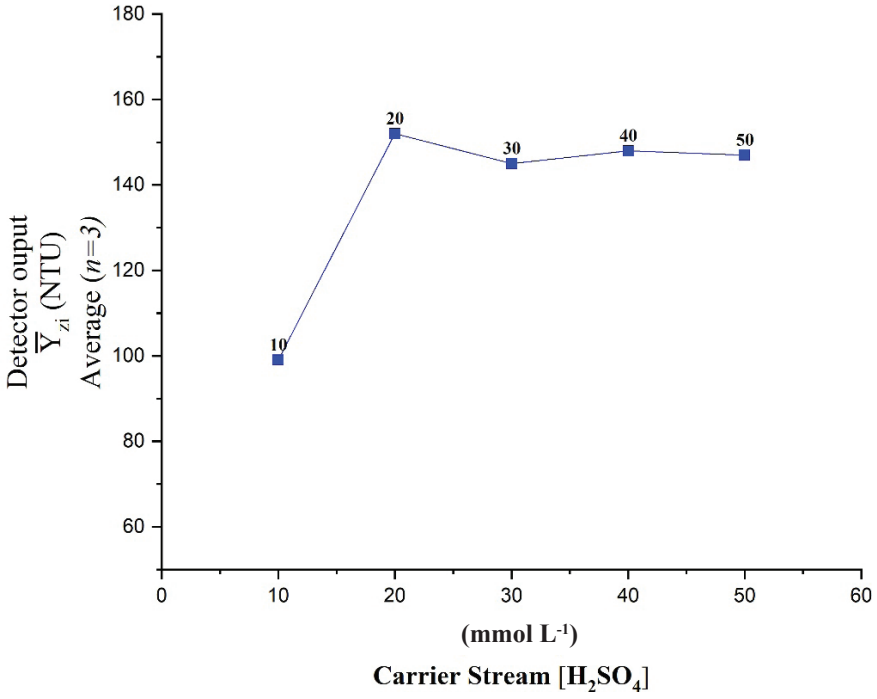

Fig.7. Optimization the concentration of sulfuric acid

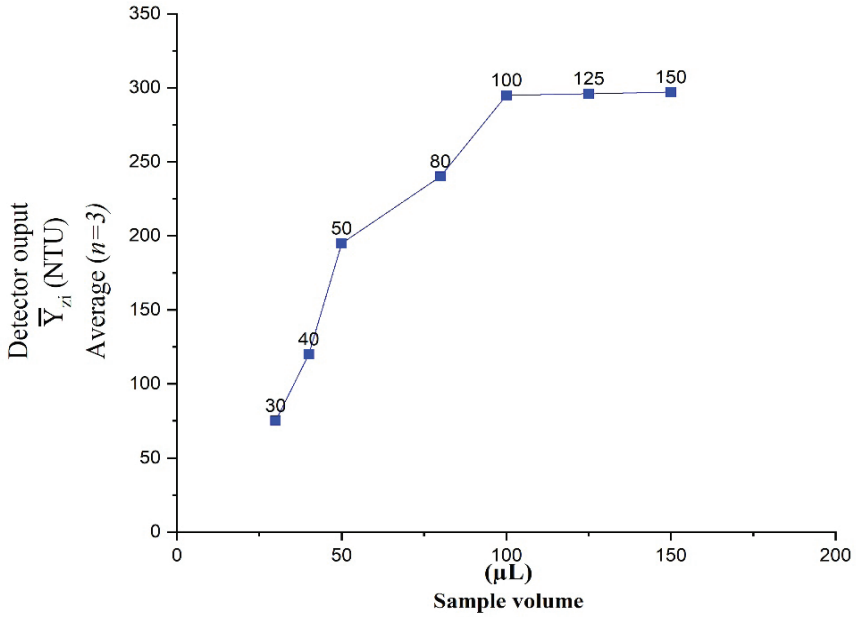

Fig. 8. The effect of the injected sample volume of promethazine [µL] on the determination of promethazine using experimental conditions: flow rate of lines 1.0 ml min^{-1}, STPB (30 µg mL^{-1}) and open valve mode.

Figure 8, 100 µL was the ideal sample segment. One of the most common physical characteristics that need to be optimized in any flow system is the flow rate. Therefore, flow rates of 0.2-4.0 mL min^{-1} were used to propel the two lines (carrier stream and reagent line). The greatest signal was attained at 2.0 mL min^{-1} based on the acquired data, as shown in Figure 9. As a result, this flow rate of 2.0 mL min^{-1}

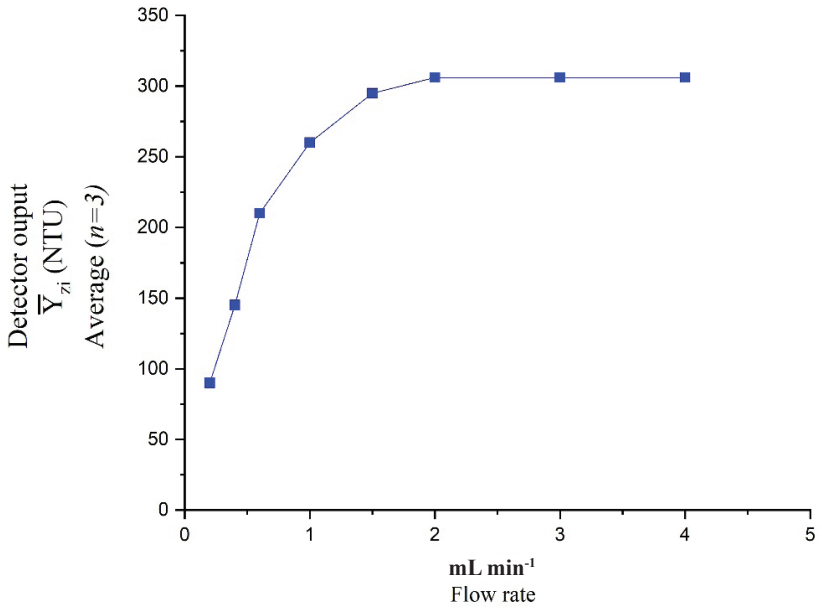

Fig. 9. The effect of the flow rate of lines [ml min^{-1}] on the determination of promethazine using experimental conditions: 100 µL of promethazine (50 µg ml^{-1}), STPB (30 µg ml^{-1}) and open valve mode.

was selected for use in further experiments.

Finally, to guarantee that the reaction between the promethazine and the organic reagent (STPB) was complete, mixing coils of various lengths (0–20 cm) were used. The highest-profile signal was seen at a distance of 10 cm; however, using mixing coils longer than 10 cm caused the peaks to spread, which is not desired in flow systems. As a result, the ideal mixing coil is 10 cm, which was employed in further studies as shown in Figure 10. Table 1 shows the optimal concentration ranges for all physical and chemical characteristics

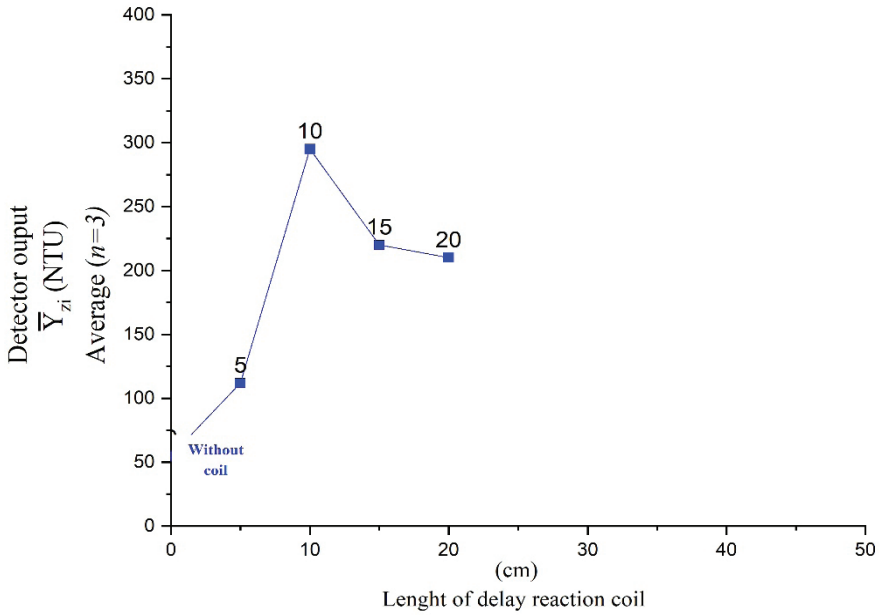

Fig.10. The effect of using variable mixing coil lengths [cm] on the determination of promethazine using experimental conditions: flow rate of lines 2.0 mL min^{-1}, open valve mode STPB (30 µg mL^{-1}) and sample volume (100 µL).

Table 1. The optimum flow characteristics for the determination of Promethazine

Studied flow parameters	Applied Ranges		Optimum value
	From	To	
STPB concentration (µg ml^{-1})	0.5	50	30
H_2SO_4 concentrations (mmol L^{-1})[a]	10	50	25
Flow rates (mL min^{-1})[b]	0.2	4.0	2.0
Mixing coil length (cm) [b]	without	20	10
Sample volume (Injected (µL)) [b]	30	150	100

[a] The carrier streamline that carries the sample segment into the manifold system
[b] The flow rates of both lines (carrier and reagent)
[c] This part is placed after the Y-junction point to ensure the reaction is completed

examined.

The following values were chosen for flow injection parameters: STPB 30.0 µg mL^{-1}, sulfuric acid 20 mmol L^{-1}, flow rate 2.0 mL min^{-1}, 100 µL injected-sample volume and 10 cm mixing coil. With this setup and to generate the analytical curves, a series of standard promethazine hydrochloride concentrations were run in triplicate. The results for the 0.5-90 µg mL^{-1} range are illustrated in Table 2. The linearity range of the flow system integrated turbidimetric method was 0.5-90 µg mL^{-1}. The statistical examination of the linear range regression equation revealed low values of RSD %, slope (b), and high values of (r) with a detection limit of 0.35 µg mL^{-1}. Table 2 summarizes the statistical studies of the calibration curve.

The flow system repeatability was examined for promethazine hydrochloride solution containing two concentrations 20 µg mL^{-1} and 50 µg mL^{-1}, across 5 injections for each concentration resulting in a total of 10 determinations (Table 3). The relative standard deviations for the turbidimetric measurements were less than 2.0 %. And it was within the acceptance limit of ICH (The International Council for Harmonisation of Technical Requirements for Human Use) and the FDA (Food and Drug Administration) [32]. The limited sample throughput, i.e., 50.0 determinations per hour, was not accounted for by the cell washing time; however, this number may be increased by running at greater flow rates, with a minor loss in

Table 2. The developed method's statistical description of the linear equation for determining Promethazine

Linear equation Parameter	Promethazine
Linearity (µg ml^{-1})	0.5-90
Linear Equation	139.61129.5441+4.1325± C[a]±0.5221
Intercept	139.6112±9.5441
Slope	4.1325 ±0.5221
r[b]	0.9967
R^2 [c]	0.9935
LOD (mmol L^{-1})[d]	(0.35 µg ml^{-1})
% RSD [e]	Less than 2.0

[a] The detector signal in NTU for the concentration of C µg ml^{-1}
[b] Correlation coefficient
[c] Coefficient of determination
[d] The limit of detection
[e] The relative standard deviation percentage for *n=3*

Table 3. The results of the proposed method's repeatability assay for determining Promethazine

[Promethazine] (µg ml^{-1})	Detector signal in NTU	RSD %
20	230.552	0.52
50	271.225	0.19

sensitivity of the absorbance peak heights.

3.2. Applications

Promethazine hydrochloride was successfully determined in pharmaceutical preparations such as Histazine tablets (25 mg/tablet) and Phenergan tablets (25 mg per tablet) using the proposed method. Table 1 summarizes the optimal assay conditions for promethazine. The analyte was quantified using typical calibration curves in a defined concentration range and the method of standard additions for both commercial tablets. The turbidimetric data has a linear response and a decent curve fitting through the same equation that was observed in the calibration curve of standard material. The results are described in Tables 4 and 5, which also include a comparison of the suggested and official spectrophotometric (measuring the absorption band at 297 nm) methods for determining promethazine in Histazine tablets (25 mg per tablet) and Phenergan tablets (25 mg per tablet). The two sets of data are nearly identical and are consistent with the nominal value (25 mg per tablet). The value of promethazine is determined by the manufacturer during manufacturing and quality control (to improve shelf time, this medicine is routinely produced with a 10 % excess of active pharmaceutical ingredients). The manufacturer employs a time-consuming multi-step extraction procedure followed by spectrophotometric analysis of the promethazine absorbance peak at 297 nm. It really should be obvious that the novel flow turbidimetric approach is quicker and does not

Table 4. Assay results for Promethazine determination in real commercial samples using official and developed methods

PCP	VL (mg)	t[a]	F[a]	Found [b] *(n=3)*	
				Developed method	**Official method**
				FIA-turbidity	**UV λmax $_{297}$**
Material A	25	0.521	0.955	25.022±•‚12	25.122±•‚32
Material B	25	1.231	1.655	24.9550.024±	25.2441±•‚•5

PCP: Promethazine Commercial product
VL: Claimed label
Material A: Histazine 25 mg, ® United pharmaceutical, Jordan
Material B: Phenergan 25 mg ® (GLOBAL manufacturer Sanofi United pharmaceutical, UK)
[a] the tabulated values of F and t at P=0.05 (95%) are (2.168) and (2.093) respectively. [33]
[b] mean test % of label claimed ± SD

Table 5. The recovery results achieved by applying the standard addition method to the developed method

Promethazine Preparations	Promethazine in solution (mg)	Added (mg)	Total found (mg)	Recovery % [a] (mg)
Material A	25.022	5	30.225	100.61
Material A	25.022	10	36.233	100.58
Material B	24.955	5	29.995	100.13
Material B	24.955	10	35.245	100.82

[a]Average of three Promethazine determinations
Material A: Histazine 25 mg, ® United pharmaceutical, Jordan
Material B: Phenergan 25 mg ® (GLOBAL manufacturer Sanofi United pharmaceutical, UK)

require any prior extraction processes.

4. Conclusion

Because the manifold system can perform 50 tests per hour, the design of this flow cell boosts its throughput, making it an effective instrument for routine analysis of active pharmaceutical ingredients. Using the design flow cell can increase the speed of measurements, minimize the number of chemical tools that must be ordered, cleaned and stored, and finally, eliminate the need for matched sample cells. The developed method for determining promethazine hydrochloride in pure form or in pharmaceutical dosage forms is straightforward, quick, and accurate. It's a great illustration of the turbidimetric approach's potential and attractiveness for analytical applications, either alone or in tandem with FIA techniques. The combined approaches (FIA and turbidity measurements) help to increase method validity by allowing for analysis of the sample in a very short time (one minute), which is important in increasingly complicated samples. Therefore, as a rule, the interfering compounds included in this sort of matrix, such as excipients (sugar, propylene glycol, dyes, ascorbic) usually do not create difficulties. Promethazine analytical recoveries varied from 100.13 to 100.81 %. At the 95 % confidence level, the levels of promethazine estimated by the proposed approach and amounts obtained by the official method were not considerably different. The proposed approach is low-cost, simple, and safe to use, uses little sample and reagent, and allows for quantitative measurement of the target analyte in both pure and dosage forms. The proposed flow cell is acid-resistant and does not require special care by the analyst. So, the presented technique has better sensitivity and the capacity to perform the reaction under the high acidity of the reaction medium. The apparatus is readily available in any analytical laboratory, and the manifold system cell may be constructed from affordable components, resulting in little reagent usage.

5. Funding and Conflicts of Interest

This work was supported by the authors and the authors declare no conflicts of interest

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7. References

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