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DESIGNING OF THE FLOW INJECTION SYSTEM TO DETERMINE OF GLUTATHIONE DRUG BY DTNB

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Abstract: In this study, the stopped-flow injection technique was used because it is considered one of the important, accurate high-speed techniques in giving results and examining a large number of samples in a very short time. Because glutathione is an important drug that contains a thiol group, which is used in the treatment of cancer, liver and heart diseases, Alzheimer's, and others, it was estimated in pharmaceutical preparations by reacting with the DTNB reagent in the presence of a Na2HPO4 buffer solution using the above-mentioned technique, and a yellow product is formed at a wavelength of 417. Nanometer and study of the physical and chemical properties of the reaction including flow rate 1.5 ml/min while reaction coil 75 cm in a time not exceeding 20 seconds at a temperature of 40 C^0 , The detection limit and quantitative limit were 0.23 and 0.766 for the concentration rate 25-250 ppm and the SD was 0.2182 while the RSD % = 20.98, and R^2 = 0.9964.

Keywords: flow injection, DTNB, Glutathione.

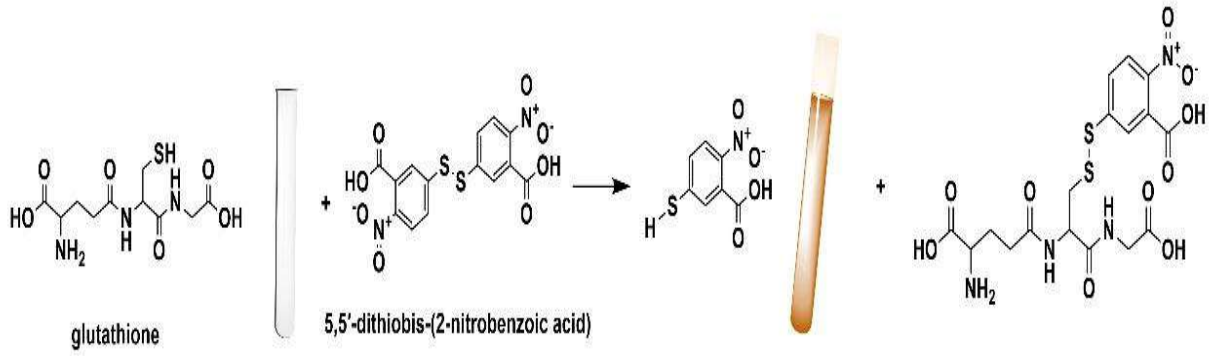
1. Introduction

Flow injection technique restricts sample longitudinal dispersion along the flow stream, which minimizes sample contact and allows for a more extended residence period of the sample, hence boosting sensitivity and allowing for comparatively slow reactions (1). A continuous flow injection analysis (CFIA) manifold injects a liquid sample into a non-segmented continuous carrier stream of a suitable liquid using a (CFIA) manifold. This equipment is simple, affordable, and has a high sample rate and dependability while still being easy to use(2). The residence time of the model in a non-segmented stream is much less than the residence time in an air-segmented system (3).

Several attempts have been made, and many efforts have been made to develop the technique of traditional normal flow injection (nFIA) injection analysis, which resulted in the emergence of several forms of this technique, including injection and stopped-flow injection analysis (rFIA), stopped-flow and confluent areas, Merging zone and others (4).

Two different flow stop models can be used in the stop-flow technique: Turning off the pump significantly reduces the modeling speed d because the system needs a higher flow rate to restore the balance after detecting each sample, Diverting the flow from the detector flow cell during the measurement period without stopping the pump by placing a three-way valve between the injection

point and the detector flow cell or using two injection valves to hold the injected sample and the used detector(5),as shown in fig [1]



Fig[1]:Reaction Glutathione with DTNB

2. Materials and Methods

2.1. Chemicals: To complete the project with accurate and correct results, chemicals were used in their pure form from well-known and registered companies. Glutathione was used in its pure form (Thomas Baker (Chemicals) Pvt. Ltd.), Elmans reagent DTNB (Thomas Baker (Chemicals) Pvt. Ltd.), and buffer solution Na_2HPO_4(Thomas Baker (Chemicals) Pvt. Ltd.).

2.2. Instrument

Several devices were used: Peristaltic pump, Rotary 6- port injection valve, Competence Analytical Balance, Biologic Quad Tec UV-Vis Detector.

2.3. Preparation of Glutathione

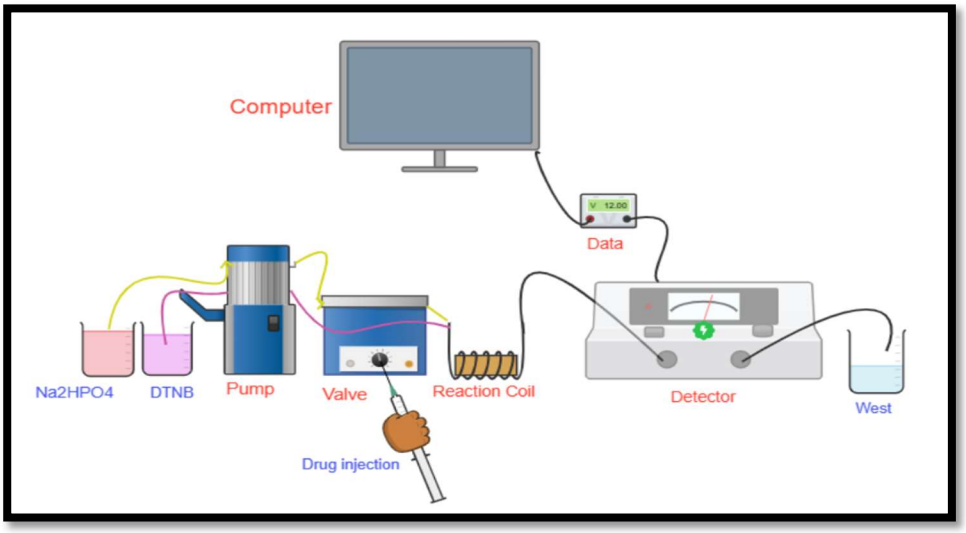
A standard drug was prepared by dissolving 25 mg (0.025 g) of Glutathione in 25 mL of distilled water and diluting it to 100 mL in a volumetric flask with distilled water.

2.4. Procedures

DTNB solution: The standard reagent solution was prepared by dissolving 0.1g of sodium citrate (100 mg) in 25 ml of distilled water and adding 0.050 g(50 mg) of DTNB to it, and placed in a shaker for 10 seconds to ensure the dissolution of the DTNB granules and then diluted to 100 ml in a beaker My volume with distilled water(6).

Na_2HPO_4: The material is prepared by weighing 200 mg and dissolving it in 100 ml to obtain a concentration of 2000 ppm.**Design of Flow Injection Units**

Continuous flow injection analysis systems consist of the basic parts: the pump system for pumping liquids, the reaction coil, measuring devices, the reading system, and the injection unit as shown in **Scheme [1]**



Scheme [1]: Flow injection unit design.

3. Results and discussion

3.1. Peristaltic Pump

The first stage begins with the operation of the cylindrical multichannel peristaltic pump.
The pump has a different speed as each speed gives a certain and constant volume of the conveyor solution per minute that can be controlled, as shown in the following tab1.

Tab 1: The amount of flow velocity per velocity of the pump

Flow rate (mL/min)	The volume descending from the system(mL) in one minute	Speed recorded on the pump
0.5	0.5	1
1.0	1.0	2
1.5	1.5	3
2.0	2.0	4
2.5	2.5	5
3.0	3.0	6
3.5	3.5	7
4.0	4.0	8
4.5	4.5	9
5.0	5.0	10

3.2. Unit Injection

The second stage involves This stage is represented by the entry of the reagent or sample into the loop of the injection unit, as the direction of filling the reagent connection or the sample is from one external movement. Change in the direction of the valve leads to the flow toward the reaction

coil as the materials mix. Reaction coil It is the unit in which the mixing of all reaction materials occurs, The volume of the carrier solution carried by the loop can be calculated through the following equation.

$$v=r2\pi h$$

Tab 2. The size of each interaction coil

Volume (µL)	Length of reaction coil (cm)
196.25	25
392.50	50
588.75	75
785.00	100

3.3. Determine the maximum wavelength (λ max)

Gluathione's maximum absorption wavelength with a DTNB reagent was scanned using a UV Vis spectrophotometer. A UV-Vis spectrum of DTNB solution showed a maximum absorption peak at 412 nm, Adding an appropriate volume from the previous solution in a convenient concentration to the alkaline Gluta solution will produce a Yellow reaction product with a new characteristic peak(7) as shown in **Fig [1]** .

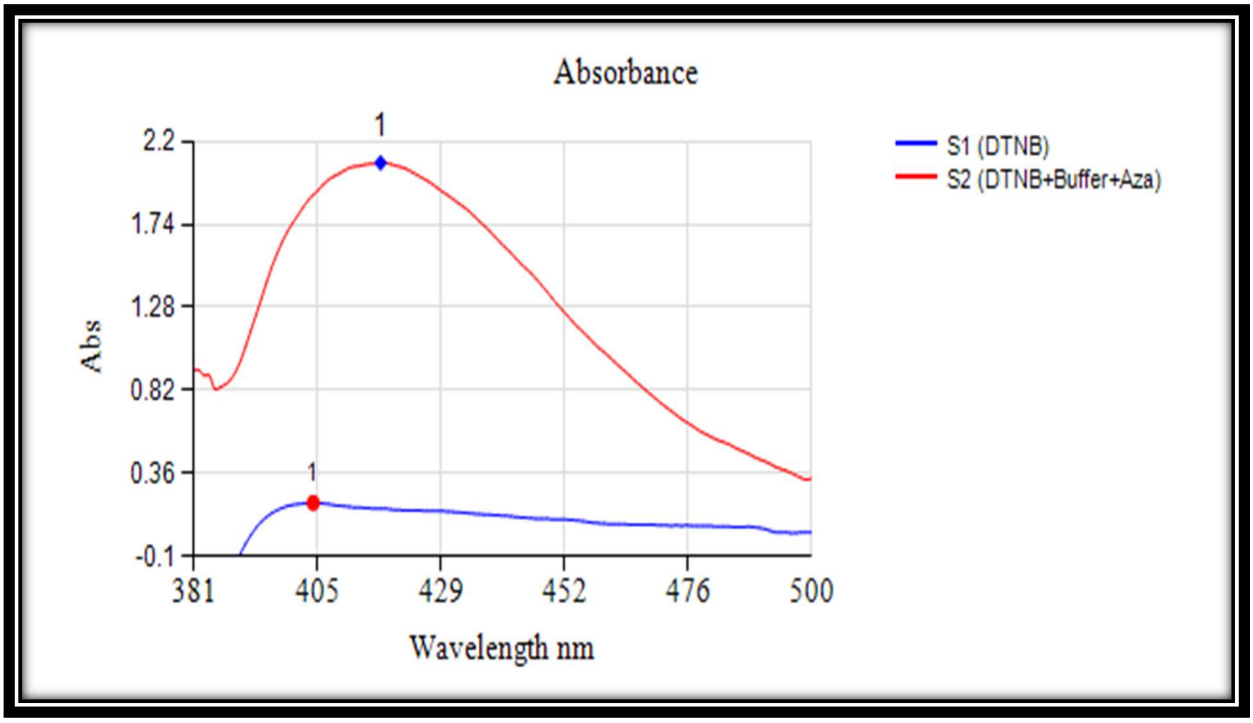


Fig [2]: UV- absorbance scanning spectrum for the reaction product between 10 ppm Gluta and 10 ppm DTNB with $Na_2HPO_4$3.5. Determine the appropriate Flow Rate

The peristaltic pump contains different speeds starting from 1 to 50. Each speed gives a certain volume of the carrier solution in it, where the flow rate (1,2,3,4,5) was used to give

(0.5,1.0,1.5,2.0,2.5) ml/min on respectively; when the drug is injected into the valve (0.001), and each of the reagent solution DTNB (40 ppm) and buffer solution (2000 ppm) are made, the carrier solutions pass through the pump and mix in a reaction coil with a length of (25) cm at wavelength (413)nm and the stopping time is 3 minutes, as shown in the figure[3.6]. Through the obtained results, we find that the first speed, 0.5 ml, has a higher absorbency, but it takes a very long time compared to the 1.0 and 1.5ml/min, while we find that the 2.0 and 2.5 ml/ min and fifth speeds have little sensitivity due to the speed of the solution passing through the detector(8).

Therefore, the best reaction speed was chosen at 1.5 ml/min to have a clear peak with high absorption without dilution or dispersion in the reaction.

We conclude from this that at the highest flow rates, the analyte does not have sufficient residence time for effective reactions with sorbents, but at low Flow rates, decreased absorption efficiency, which can be attributed to adsorbed bsorption(9), as shown in **Fig [2]**

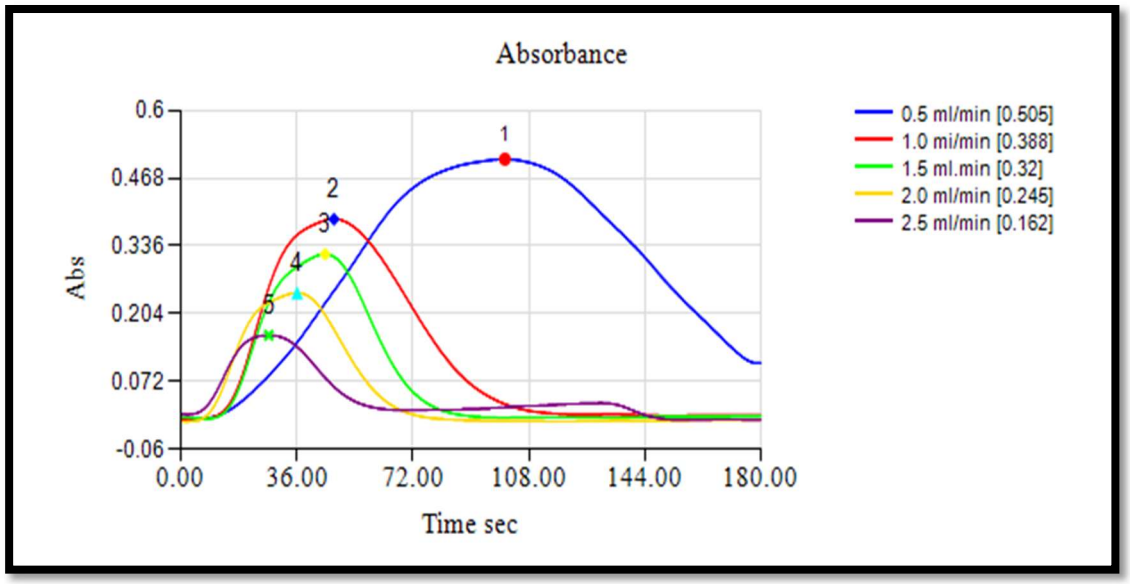


Fig [3]:Shows the effect of flow rate on the absorption peak height when Gluta conc. = 225 ppm, DTNB conc. = 40 ppm, reaction coil= 25cm, used buffer solution Na_2HPO_4 =2000 ppm, at room temperature.

3.6. Determine the Time of stop The reaction was studied in different packages (0, 10, 20, 30, 40) seconds to determine the best time for the reaction to reach its maximum peak. When passing the reagent solution DTNB (40 ppm) and the buffer solution Na2HPO4 (2000 ppm) and injecting the drug (225 ppm) into the system at a wavelength of (417 nm) using a reaction coil of (50 cm) and using a flow rate(1.5 ml /min), as shown in the figure[3.7]. The reaction reached a stable state at (20, 30, 40) seconds. Choosing the best time for the interaction is 20 seconds, as it reaches the highest stage of stability, as shown in **Fig [3]**

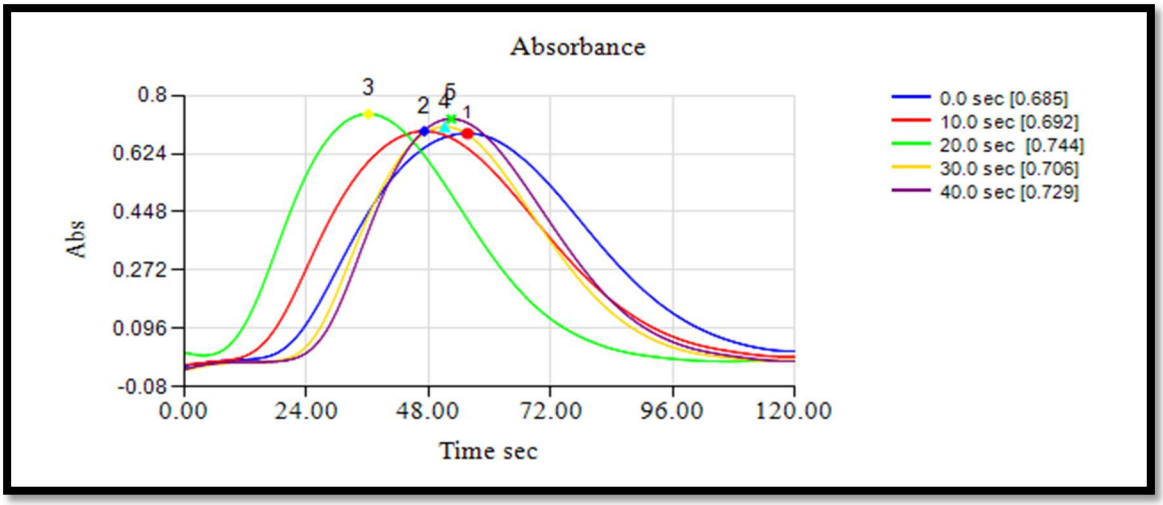


Fig [4]:Effect of time on the reaction of Gluta with the reagent DTNB at wavelength 417 nm.3.8. Determine the Length reaction coil of Glutathione with DTNB

The effect of the length of the reaction coil on the mixing process between the Gluta (225 ppm) with DTNB (40 ppm) and buffer solution Na_2HPO_4 (2000 ppm) at room temperature (25 ^{0}C) and flow rate of (1.5 ml/min) and a wavelength of (417 nm) is studied and a stop time of 20 seconds to obtain the highest mixing process, as different lengths of the reaction coil were used, starting from (25 cm, 50 cm, 75 cm, and 100 cm.) We notice that the greater the length of the reaction file, the greater the mixing process between the reaction materials without any dilution or dispersion of the material(10). The shown in Fig [4]. We find that the best reaction file in which mixing occurs and gives it high absorption and a clear peak without dilution or dispersion of the reaction is at 75 cm, as a high mixing process characterizes it and gives a clear peak with a high height free of anydispersion and does not need a long time as It is in the reaction coil 100 cm.

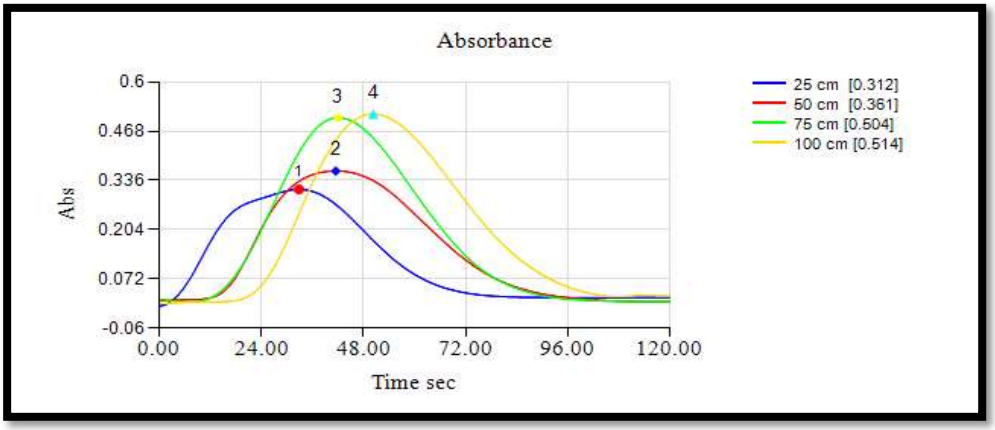


Fig [5]: This shows the effect of the reaction coil on the absorption peak height when Gluta conc. = 225 ppm, DTNB conc. = 40 ppm, Na_2HPO_4 =2000 ppm at room temperature.

3.9. The effect of temperature on the interaction of the Gluta with DTNB

The experiment was conducted at different temperatures starting from 30, 35, 40, 45, and 55 degrees Celsius to find out the best temperature at which the reaction can reach the peak without any breakage or disintegration of the reaction under the following conditions: Gluta=225 ppm, DTNB=50 ppm, Na2HPO4=2000 ppm, wavelength = 417nm, using the reaction coil = 75 cm, with a stop of time 20 seconds and flow rate 1.5 ml/min, as shown in the **Fig[5]**

Despite the difference in temperatures, there was no significant difference in the absorption of the reaction; we conclude from this that the reaction needs high temperatures to reach its peak; it can occur at 35 C^0, so the best temperature that can be used for the reaction was chosen and obtain clear peaks and high absorption free of duplication is 35 C^0.

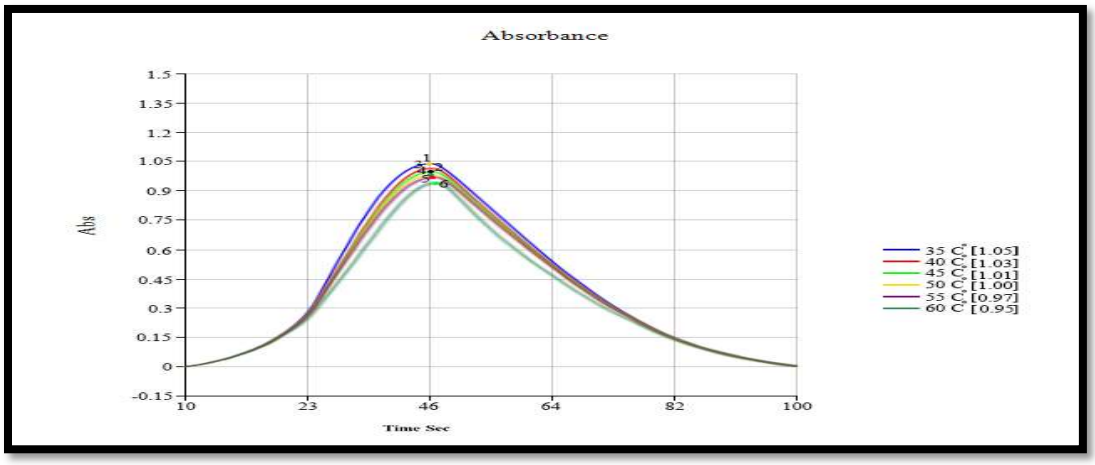


Fig [6]: Effect of temperature on the reaction of Glutathione and DTNB in the presence of Na_2HPO_4

3.10. Effect of DTNB concentration

The effect of the reagent concentration on the reaction was studied by using different concentrations (10, 20, 30, 40, and 50) ppm under the following conditions: Gluta concentration 225 ppm, the concentration of Na2HPO4 2000 ppm, wavelength = 413nm, using the reaction coil = 75 cm, with a stop of time 20 sec and flow rate 1.5 ml/min, so it was found that the best concentration of the reagent is 50 ppm, as shown in the **Fig [6]**

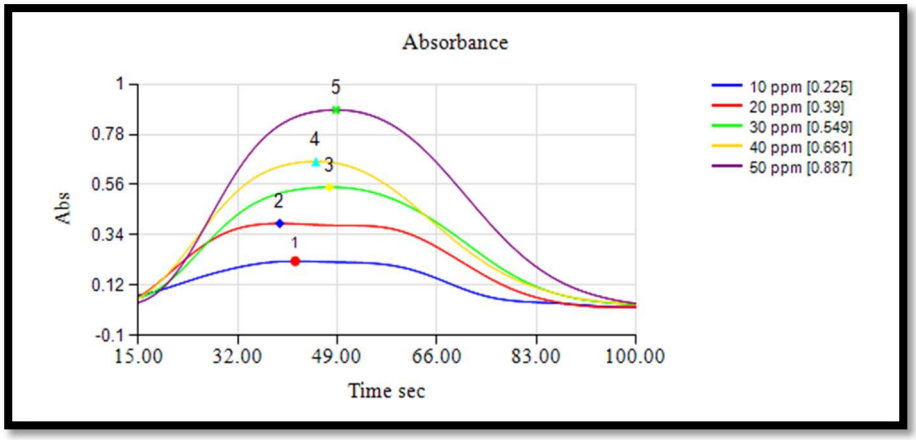


Fig [7]: The influence of DTNB concentration on the absorption peak height when Gluta conc. = 225 ppm, reaction coil length = 75 cm, temperature=35 C^0, flow rate =1.5 ml/min, stop time =20 sec.

3.10. Effect of Na_2HPO_4 concentration

The effect of the buffer concentration on the reaction was studied by using different concentrations (100,250,500,1000.1500,2000 and 2500) ppm under the following conditions: Gluta concentration 225 ppm, concentration of DTNB=50 ppm, wavelength = 417nm, using the reaction coil = 75 cm, with a stop of time 20 sec and flow rate 1.5 ml/min, Clear results were obtained from the variation and difference from as shown in the following **Fig [7]** to the difference in the concentration of the puffer, where we note that the greater the concentration of the buffer, the greater the response, and thus we get clear and high altitude single peaks free of distortion and free of double heights, and this is evidence that the reaction is free from any dispersion, cracking or disintegration of the interacting valley(11).

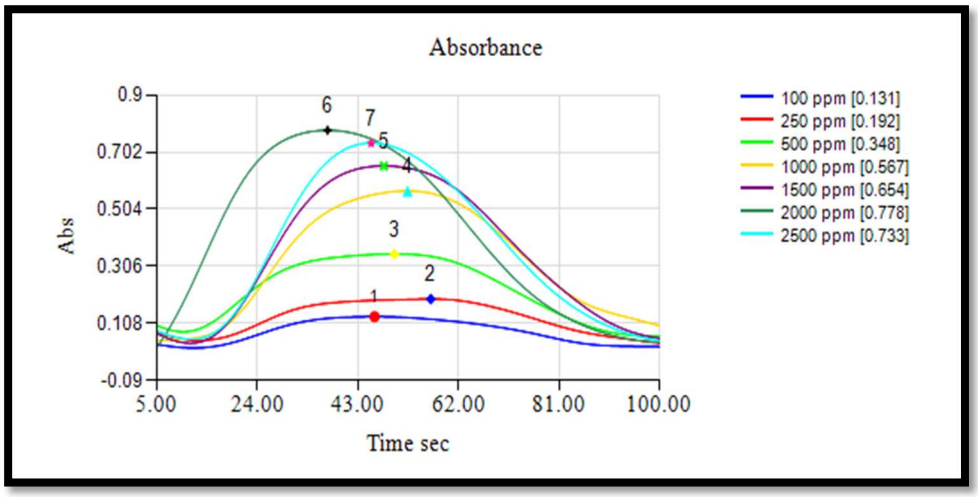


Fig [8]: The influence of Na_2HPO_4 concentration on the absorption peak height when Gluta conc. = 225 ppm, DTNB=50ppm, reaction coil length = 75 cm, T=35 C^0, flow rate =1.5 ml/min,stop time =20 sec.

According to the optimum conditions, Gluta was quantitatively determined. The calibration curve was prepared at 417 nm by organizing a series of Gluta solutions with different concentrations and analyzing each employing the FIA system. The absorption peak height value of the formed complex was plotted against the concentration. The suggested method allows for the determination of Aza in the range of (25-250) ppm.as shown in the following **Fig[8] and Fig[9]**

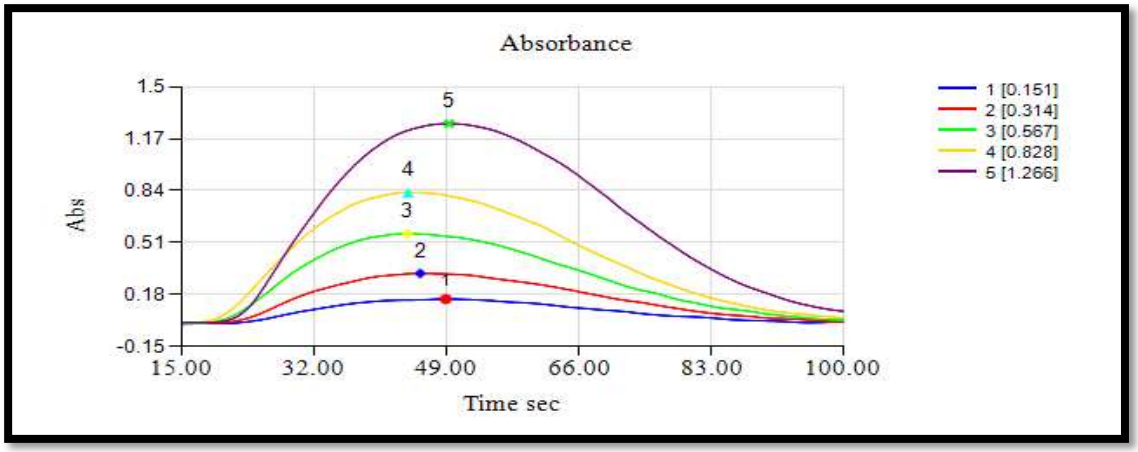


Fig [9]: Absorption spectra of Glutathione

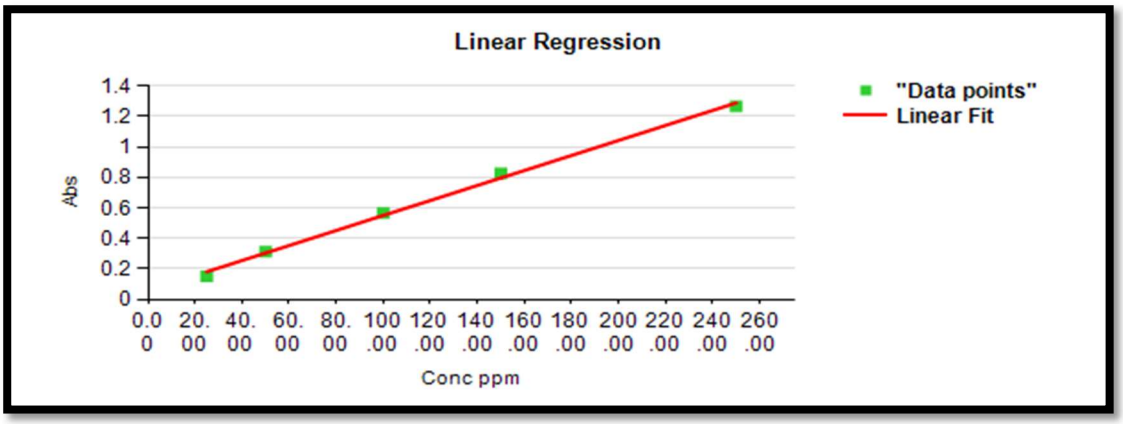


Fig [10]: Calibration curve of Glutathione

3.11. In aqueous solutions, Glutathione can be determined as follows

Three aqueous solutions were prepared and considered solutions of unknown concentration; then, the absorbance was measured according to the optimum conditions, as shown in Fig. [10].

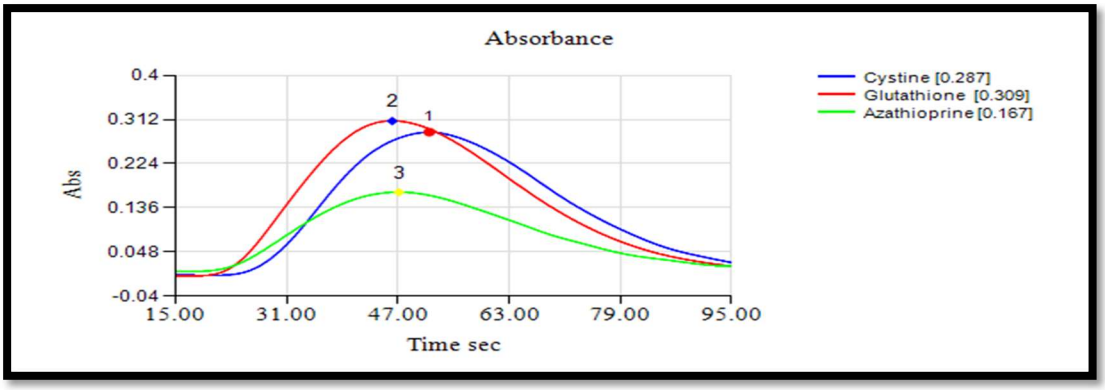


Fig. [11]: The spectrum of possible applications.

Then the solutions' concentration was determined by setting each solution's absorbance on the straight line of the previous calibration curve, as shown in **Fig. [11]**

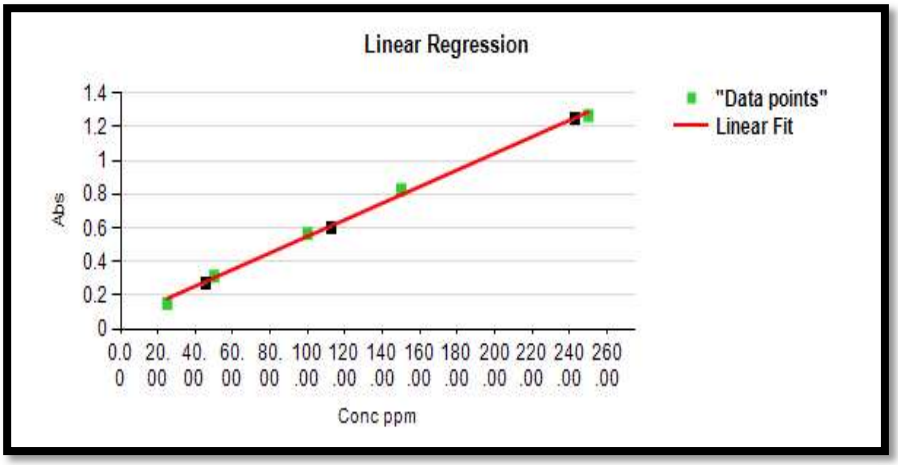


Fig. [12]: Determination of unknown aqueous solutions.3.12. The determination of dispersion

The dispersion coefficient (dilution factor D) was studied to illustrate the dispersion process extent within the flow injection system from the injection point at the valve to the detecting point at the detector(12).

In this experiment, the dispersion in the reaction is calculated, where two experiments were conducted, the first step was mixing the reaction materials in an external baker and passing them in the pump as a carrier solution, but in the dual experiment, the reagent DTNB and Na_2HPO_4 are passed as a carrier solution. Gluta is injected into the pump; we notice the height of the peak and the high response when the reaction reaches the Detector, as shown in the following figure. as shown in the following **Fig [12]**

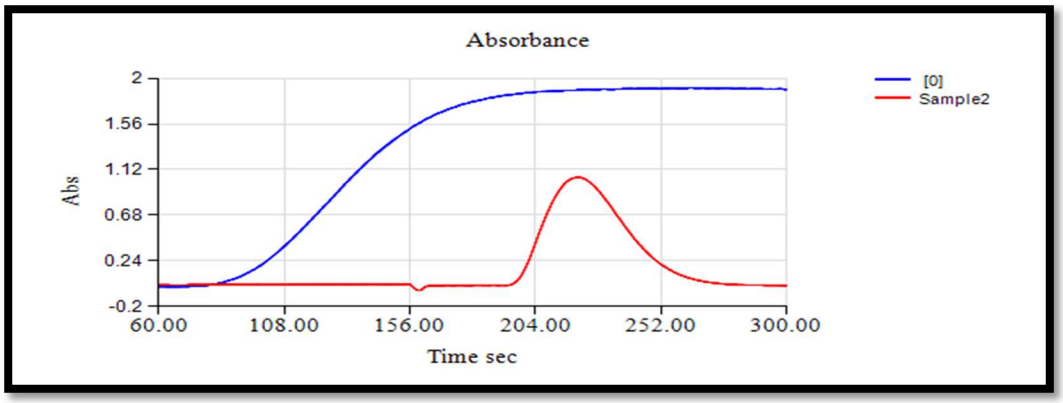


Fig [13]: Dispersion coefficient study results for Gluta determination FIA system

3.13. Repeatability

The relative standard deviation (RSD %) representing the precision of the proposed method has been studied utilizing 150 ppm of Gluta solution. At the optimum conditions, 6 replicate

measurements were carried out for the determined concentration and recorded as shown in Fig. [13] and Table [3]. The standard deviation value is equal to 0.0019, and the relative Standard deviation value is equal to 1.237, indicating the high precision of the suggested method.

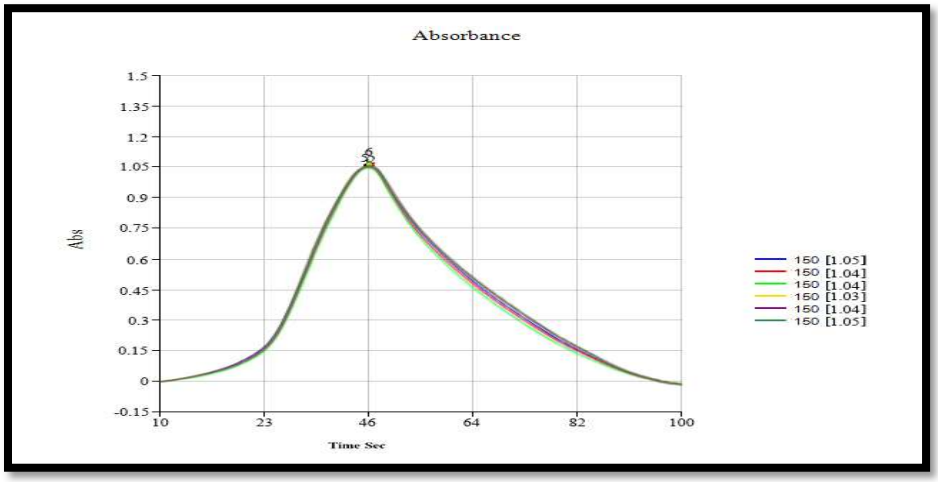


Fig [14]: Repeatability of 150 ppm for Gluta solution by FIA

Table [3]: Repeatability results for 150 ppm Gluta solution by FIA

Sample No.	1	2	3	4	5	6	Mean	SD	RSD%
Flu.	1.05	1.04	1.04	1.03	1.04	1.05	1.04	0.2182	20.98

Conclusion

1. Drugs can be identified in the presence of reagents of thiol groups under study using the flow injection technique. The study showed that all the designed systems are highly effective.
2. Estimation of Gluta in the presence of the detector (DTNB) in the flow this technique is characterized by high speed, accuracy, sensitivity, and responsiveness in the analysis and estimation of drugs with the lowest concentrations, the largest number of samples, and the smallest volumes of reagents in a very short time. Due to these characteristics, it has made it one of the preferred techniques in analytical chemistry.
3. The flow injection system is characterized by not consuming chemicals in large quantities, as it is characterized by the use of tiny volumes and low concentrations of both the reagent and the carrier solution mixture, as well as the small sample volume.
4. Other benefits of the new systems were high selectivity and recovery, good repeatability, fair relative standard deviation values, high sampling rate, good precision and accuracy.
5. Determine the linear range of the calibration graph for the reaction of the reagent DTNB with Glutathione and their determination in aqueous solutions.

References

1. Ruzicka J, Hansen EH. Flow injection analysis: John Wiley & Sons; 1988.
2. Ruiz-Capillas C, Jiménez-Colmenero F. Application of flow injection analysis for determining sulphites in food and beverages: A review. Food chemistry. 2009;112(2):487-93.
3. Trojanowicz M, Kołacińska K. Recent advances in flow injection analysis. Analyst. 2016;141(7):2085-139.
4. Kolev SD, McKelvie ID. Advances in flow injection analysis and related techniques. 2008.
5. Trojanowicz M. Flow injection analysis: instrumentation and applications: World Scientific; 2000.
6. Betteridge D. Flow injection analysis. Analytical Chemistry. 1978;50(9):832A-46A.
7. Giné M, Zagatto E, Reis B. Simultaneous determination of nitrate and nitrite by flow injection analysis. Analytica chimica acta. 1980;114:191-7.
8. Raju CV, Rani GM, Haribabu J, Kumar SS. Flow injection analysis-based electrochemiluminescence: an overview of experimental design and its biosensing applications. ECS Sensors Plus. 2022;1(3):031604.
9. Al-Taai, S.H.H.; Kanber, H.A.; al Dulaimi, W.A.M. The Importance of Using the Internet of Things in Education. Int. J. Emerg. Technol. Learn. 2023, 18, 19
10. Mohammed, A., Al-Saadi, H., & Al-Taai, S. (2022). Information Sources and their Role in E-learning from Iraqi College Students' Viewpoint. Webology, 19(1), 1128-1150. https://doi.org/10.14704/WEB/V19I1/WEB19077
11. Sornambigai M, Venkateswara Raju C, Senthil Kumar S. Bimodal electrogenerated chemiluminescence of the luminol/dicyclohexylamine (DCHA) system: a novel and highly sensitive detection of DCHA via ECL-flow injection analysis. Analytical Chemistry. 2021;93(16):6472-80.
12. Wang Z-H, Zhang J-X, Gao M, Cui W-Q, Xu L, Zhu X-l, et al. Stable isotope labelling-flow injection analysis-mass spectrometry for rapid and high throughput quantitative analysis of 5-hydroxymethylfurfural in drinks. Food Control. 2021;130:108386.
13. Bezerra MA, Santelli RE, Lemos VA, dos Santos Alves JP, Braz BF, Santos LB. Strategies to make methods based on flow injection analysis greener. CLEAN–Soil, Air, Water. 2020;48(7-8):2000007.
14. Wang W, Ding S-N, Chen F-F, Zhang Q. Electrochemical paper-based analytical device for flow injection analysis based on locally enhanced evaporation. Sensors and Actuators B: Chemical. 2022;371:132517.