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Batch and merging-zone flow injection methods for determination of tetracycline hydrochloride

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A B S T R A C T

The objective of the present work is to develop batch and merging-zone flow injection methods for sensitive and accurate spectrophotometric determination of tetracycline hydrochloride. The methods depend on the oxidation of the studied drug with potassium permanganate in an alkaline medium, and the absorbance of the green oxidation product was measured at 610 nm. The calibration graphs in both procedures were linear in the concentration ranges of 0.5 – 25 and 1 – 25 μg mL^{-1} using the spectrophotometric and merging-zone flow injection methods, respectively. Specific and molar absorption coefficients, limits of detection and quantification, and Sandell's sensitivity were calculated. The suggested procedures were further applied to the quantitative determination of tetracycline in pharmaceutical formulations.

1. Introduction

Flow injection analysis (FIA) is an easy and rapid technique that can be employed for unstable reactions or that do not reach the equilibrium state.[1] FIA systems have many advantages, such as the low cost of the system components, reduced consumption of reagent and sample solutions, high analytical frequency, and decreased waste generation.[2] The principal aim of different kinds of FIA techniques is to analyse the maximum number of samples using minimum amounts of sample and reagent and the spent analysis time.[3]

The molecular formula of tetracycline is $C_{22}H_{24}N_2O_8$, and it possesses a broad spectrum antibacterial effect. Tetracycline is one of the most commonly used antibiotics in animal nutrition, feed additives and veterinary drugs in food-producing animals to promote growth.[4,5] Despite the high consumption of tetracycline, only a small portion of it can actually be absorbed by humans or animals, and it is difficult to biodegrade,[6] so a large quantity of tetracycline is excreted into different environmental media, including soil, surface water, groundwater, and wastewater.[7,8] This will lead to the spread of resistance genes in the environment.[9,10] Several analytical methods have been proposed for assaying tetracycline in pure form or in drug substances by high-performance liquid chromatography,[11,12] spectrophotometry,[13,14] flow injection analysis,[15,16] chemiluminescence,[17,18] spectrofluorimetry,[19,20] and electrochemical methods.[21,22] Most of these analytical methods, except for the flow-injection methods, consume large amounts of reagent, samples and solvents solutions, and take a long time to complete the reaction, in addition to the large amounts of toxic and harmful wastes thrown into the environment.

Merging-zone flow injection method is a simple, fast, and effective technique to optimize many simple and complex classical reactions by reducing the consumption of the used solutions, analysis time reduction, and the interfering effect elimination with high reproducibility and throughput, so in the present work, simple, fast, and inexpensive batch and merging-zone flow injection methods were described for the spectrophotometric determination of tetracycline hydrochloride depending on the oxidation reaction with potassium permanganate as an oxidizing agent in an alkaline

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medium at room temperature. The batch method is easy and does not need elaborate handling of the samples and tedious proceedings for the analysis, while the merging-zone flow injection method is the optimization method for the batch method using a novel lab-made valve, laying in a newly designed flow injection system. This system is efficient, simple, and inexpensive with ease of repair and cleaning. The proposed methods were further applied to evaluate the tetracycline content in pharmaceutical formulations, with very good recovery and a sample throughput equal to 80 samples per hour for the flow injection method.

2. Results and Discussion

2.1. Absorption Spectra

A UV–Vis spectrum of aqueous $KMnO_4$ solution in alkaline medium showed an absorption band at 525 nm. The addition of aqueous tetracycline solution to this solution formed a green reaction product with a new distinctive band at 610 nm (**Fig. 1**). The new band is due to the oxidation of tetracycline by potassium permanganate in the alkaline medium and the formation of manganate ions (**Fig. 2**).

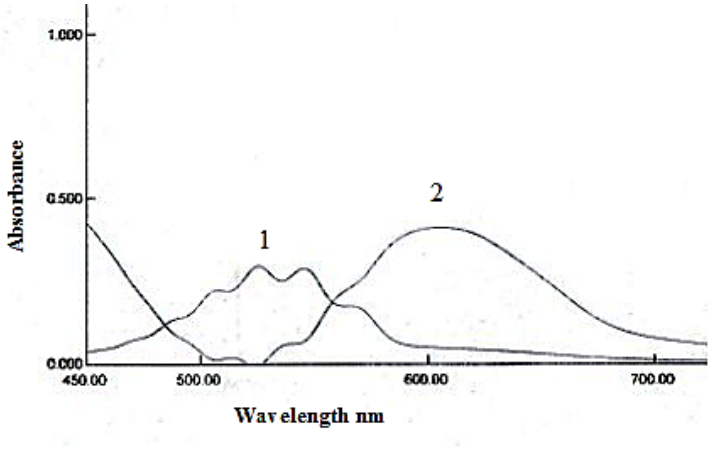

Fig. 1. Absorption spectra of (1) 6.33×10^{-4} mol L^{-1} $KMnO_4$ in alkaline medium, (2) 50 μg mL^{-1} tetracycline hydrochloride and 6.33×10^{-4} mol L^{-1} $KMnO_4$ product in alkaline medium

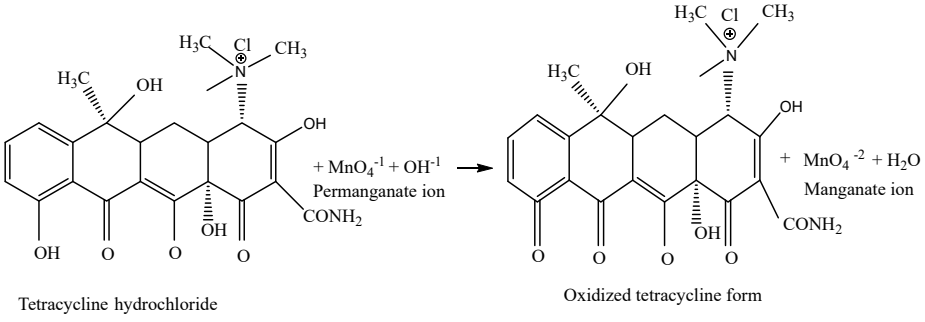

Fig. 2. The oxidation reaction of tetracycline by $KMnO_4$ in alkaline medium

2. 2. Optimization of the Reaction Conditions

2. 2. 1. For the Batch Spectrophotometric Method

The concentration and the volume of $KMnO_4$ solution, reaction time, the concentration and the volume of NaOH solution, and temperature were studied and optimized by altering each factor in turn individually while keeping the other factors constant.

The influence of $KMnO_4$ concentration on the reaction product absorbance was investigated by measuring the absorbance of solutions consisting of a fixed concentration of tetracycline (10 μg mL^{-1}), 0.5 mol L^{-1} NaOH and .٠1 mL of different concentrations of $KMnO_4$ from 1.58×10^{-4} to 31.64×10^{-4} M at a fixed time of 3 minutes. The maximum absorbance was achieved with 0.1 mL of 18.98×10^{-4} mol L^{-1} $KMnO_4$ (**Fig. 3a**), and this concentration was employed in the subsequent experiments.

The influence of different volumes (0.1 to 0.5 mL) of 18.98 mol L^{-1} $KMnO_4$ solution on the reaction product formation was also studied by measuring the absorbance of 10 μg mL^{-1} tetracycline and 0.5 mol L^{-1} NaOH solution at a fixed time of 3 minutes. The maximum absorbance was obtained when 0.2 mL of $KMnO_4$ was used (**Fig. 3b**). Thus, 0.2 mL of 18.98 mol L^{-1} $KMnO_4$ was established as the optimum volume. The effect of reaction time was studied in the range of 1-10 minutes. Although the reaction was completed in 30 minutes and this is the optimum time for the reaction between the sample and the reagent solutions to complete, 3 minutes was selected as the favorable reaction time. Certainly, this reaction time does not allow the reaction to reach the endpoint and does not give the maximum value of absorbance. But when this time is fixed in subsequent experiments to determine the optimal conditions, the reaction that occurs to a certain point will be constant and repeated in each done analysis and will not lead to an error in the measurement and calculation processes.

The influence of NaOH concentration was examined by taking 1 mL of 10 µg mL^{-1} tetracycline, 0.2 mL of 18.98 mol L^{-1} $KMnO_4$ solutions and 1 mL of varying concentrations (0.1-0.8 mol L^{-1}) of NaOH at a fixed time of 3 minutes. Maximum absorption was achieved when 1 mL of 0.5 mol L^{-1} NaOH was used. Above this concentration, no change in absorbance value could be detected (**Fig. 3c**).

The effect of NaOH volume was also investigated at a constant concentration of tetracycline (10 μg mL^{-1}), 0.5 mol L^{-1} NaOH and varying volumes (0.15–1.0 mL) of 0.5 mol L^{-1} NaOH solutions. The optimum absorption was obtained with 0.25 mL of NaOH (**Fig. 3d**). Hence, this volume was used in the subsequent experiments. The oxidation–reduction reaction was achieved at room temperature (25 ± 5 ˚C). To obtain the optimum results, the reaction was carried out in a thermostatically controlled water bath. The results showed that increasing the water bath temperature was found to produce an increase in absorbance, although at a higher absorbance, room temperature was chosen as the optimum temperature for this study because of the probability of manganese (II)

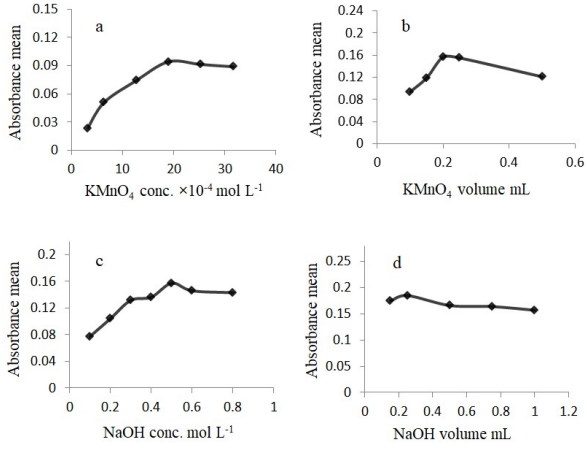

Fig. 3. Effect of different variables on the analytical signals obtained by the batch method

2. 2. 2. For Merging-zone Flow Injection Method

Preliminary experiments under merging-zone flow injection analysis conditions were carried out to examine the manifold configuration and establish the best experimental conditions. Experimental factors such as flow rate, reaction coil length, reagent concentration, NaOH concentration, reagent volume and sample volume were examined and optimized by changing each factor individually while keeping the other factors constant to achieve maximum absorption peak height, minimum dispersion and low residence time. All experiments were carried out at room temperature (25 ± 5 ˚C).

The effect of the carrier stream flow rate on the absorbance signal was examined over the range 0.8-6.2 mL $min.^{-1}$ for the determination of a 10 μg mL^{-1} tetracycline hydrochloride standard solution. The results showed that the peak height increased gradually as the flow rate increased up to 3.6 mL $min.^{-1}$ (**Fig. 4a**). At higher flow rates, a decrease in the peak height was observed, so 3.6 mL $min.^{-1}$ was chosen in the subsequent experiments. Various reaction coil lengths (10, 20, 30 and 40 cm) were tested to examine the effect of reaction coil length using a constant concentration and volume of tetracycline and $KMnO_4$, the maximum peak height was obtained at a 20 cm reaction coil length (**Fig. 4b**). At longer lengths, there was a slight decrease in the peak height; therefore, a 20 cm reaction coil length was adopted.

The influence of $KMnO_4$ concentration was investigated in the range of 1.58×10^{-4}-18.98×10^{-4} mol L^{-1} with a fixed tetracycline concentration of 10 μg mL^{-1} and the same selected variables. An increase in the absorption peak height was observed with an increase in the concentration of $KMnO_4$ up to 6.33×10^{-4} mol L^{-1} (**Fig. 4c**). The concentration of 6.33×10^{-4} mol L^{-1} was chosen subsequently. The influence of NaOH concentration as a solvent solution for tetracycline was examined in the range of 0.1-0.6 mol L^{-1} with a fixed tetracycline concentration of 10 µg mL^{-1} and the same selected

variables. A gradual increase in the peak height up to 0.4 mol L^{-1} was observed (**Fig. 4d**), so 0.4 mol L^{-1} NaOH was utilized as an optimum concentration.

The effect of the injected volume of $KMnO_4$ solutions 78.57- 235.71 µL on the height of the absorption peak was studied. It was found that the highest peak was obtained with an injected volume of 78.57 µL, and this volume was used subsequently. The effect of varying the injected volume of tetracycline solutions in the range of 117.86-314.29 µL was tested. The highest peak was obtained when the injected volume was 157.14 µL; therefore, 157.14 µL was chosen as the optimum tetracycline injected volume.

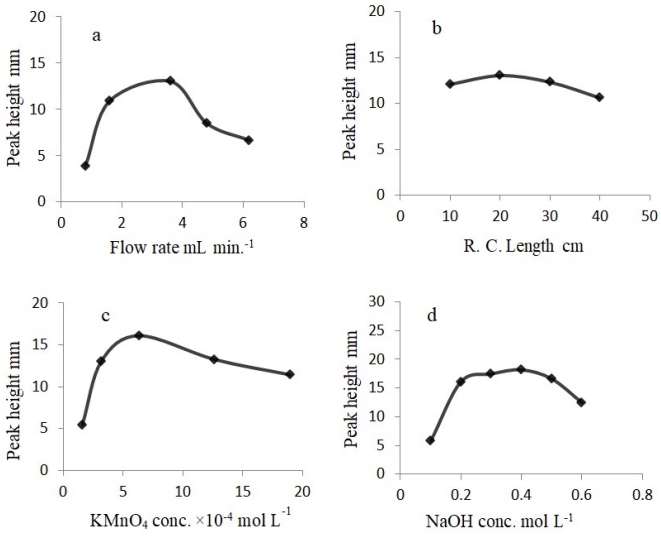

Fig. 4. Effect of chemical and physical variables on the analytical signals obtained by FIA

According to the selected experimental conditions above, approximately 45 seconds are needed for each determination; therefore, the sampling frequency for the suggested system is approximately 80 samples per hour.

2.3. Evaluation of the Proposed Methods

Under the optimum conditions for the two methods, tetracycline hydrochloride was determined, and the calibration curves were obtained at 610 nm by analyzing a series of tetracycline standard solutions in triplicate. As shown in (**Table 1**), the proposed methods permit the determination of tetracycline hydrochloride in the range of 0.5-25 µg mL^{-1} and 1-25 µg mL^{-1} for the batch and flow injection methods, respectively. This range of concentrations for determination is wide compared to other spectroscopic methods [5, 23]. The sensitivity of the proposed methods represented as the limit of detection (LOD) and the limit of quantitation (LOQ) were estimated to be approximately 0.233 and 0.419 µg mL^{-1} and 0.775 and 1.397 µg mL^{-1} for the batch and flow injection methods, respectively. The slope of the calibration curve for the flow injection method (0.0129) is much greater than the slope of the batch method (1.2449), both methods have the same good correlation coefficient value (0.9997). The values of the specific absorption coefficient and molar absorption coefficient for the flow injection method are much higher than the batch method values, while the Sandell's sensitivity value for the flow injection method are much lower than the batch method value. Both methods have a good recovery average, which indicates a high accuracy for the proposed methods.

Table 1. The analytical and statistical parameters for the proposed methods.

Parameter	Batch method	Merging-zone flow injection method
Beer's law range µg mL^{-1}	0.5- 25	1 - 25
Limit of detection µg mL^{-1}	0.233	0.419
Limit of quantitation µg mL^{-1}	0.775	1.397
Regression equation y = bx +a	y = 0.0129x+0.0549	y = 1.2449x+5.5034
Slope (b)	0.0129	1.2449
Intercept (a)	0.0549	5.5034
Correlation coefficient r^2	0.9997	0.9997
Specific absorption coefficient L g^{-1} cm^{-1}	12.900	$1.245\times10^{+3}$
Molar absorption coefficient Ɛ/ L mol^{-1} cm^{-1}	$6.204\times10^{+3}$	$5.987\times10^{+5}$
Sandell's sensitivity S/ µg cm^{-Y}	0.078	0.803×10^{-3}
t -test *	3.424	3.389
Percent recovery %	97.914-98.857	96.457-98.071

* Theoretical t -value at the 95% confidence level is 4.303

The precision of the methods, which is represented as the relative standard deviation (RSD %), was tested by analysing 10 replicate samples of 7 µg mL^{-1} tetracycline hydrochloride at the optimum conditions and mesuring the absorbance value (Abs.) applying the spectrophotometric method and the peak height (P. H. in mm) applying the FIA method, followed by calculating the RSD % values (**Table 2**). The RSD % values were 0.587 and 0.628 for the batch and flow injection methods, respectively indicating the high precision of the proposed methods.

Table 2. The repeatability results for 7 μg mL^{-1} of tetracycline hydrochloride by spectrophotometric and FIA methods.

Sample no.	1	2	3	4	5	6	7	8	9	10	Mean	SD	RSD %
[a] Abs. Batch method	0.144	0.143	0.142	0.143	0.144	0.144	0.144	0.143	0.144	0.145	0.144	0.001	0.587
[b] P. H. FIA method	14.15	14.22	14.38	14.36	14.22	14.15	14.22	14.20	14.38	14.22	14.25	0.089	0.628

[a] Abs. = absorbance value, [b] P. H. = peak height value (in mm)

The specificity of the proposed methods was examined by investigating the effect of excipients and additives that combine tetracycline hydrochloride in its pharmaceutical formulations, such as glucose, lactose, sucrose, starch and talc existing in certain quantities varies according to manufacturers and the different types of pharmaceutical formulations.[5, 24, 25] Synthetic standard 7 µg mL^{-1} solutions of tetracycline hydrochloride containing excipients in proportions of 1:1 and 1:10 w/w were tested for interference effects using the proposed methods (**Table 3**). By applying the spectrophotometric batch method, the interference effect study showed that starch and talc do not interfere in tetracycline determination; however, glucose and lactose had a slight effect only at 70 µg mL^{-1}.

By applying the flow injection method, the interference effect study showed that the excipient under investigation had no effect on tetracycline determination. This is due to the characteristics of the flow injection method, which is represented by reducing the volumes of solutions used during the analysis, which in turn reduces the amount of interfering influence, in addition to the main factor, which is reducing the time taken by the analysis in order to read the absorbance value of the reaction product, and this prevents the interferences from exerting their influence On the interaction of the sample solution with the reagent solution in the merging-zone in various ways. This indicates that this method is very selective for the estimation of tetracycline in the presence of different types of interference.

Table 3. The interference effect on the tetracycline hydrochloride determination applying the suggested methods

Interference type	Interference conc. µg mL^{-1}	Tetracycline conc. µg mL^{-1}	Percent Error E%	
			Batch method	Flow injection method
Glucose	7	7	0.694	0.246
	70	7	5.333	0.337
Starch	7	7	0.889	0.632
	70	7	0.778	0.702
Lactose	7	7	0.556	0.421
	70	7	4.389	0.526
Talc	7	7	0.000	0.000
	70	7	0.000	0.000
Sucrose	7	7	0.000	0.000
	70	7	0.156	0.091

The accuracy of the methods represented as recovery determinations was calculated by replicate measurements (n = 5) of 7 µg mL^{-1} tetracycline hydrochloride pharmaceutical preparations solutions. The percentage recoveries were found to be 97.914-98.857% and 96.457-98.071%, respectively, as shown in (**Table 4**) , as shown from the table results, the presence of interferences that accompanied tetracycline in the pharmaceutical formulations had no effects on the tetracycline determination by applying the suggested methods.

Table 4. Determination results of tetracycline in pharmaceuticals.

Preparation	Taken µg mL^{-1}	Batch method			Merging-zone flow injection method		
		Found µg mL^{-1}	E % [a]	Rec. % [b]	Found µg mL^{-1}	E % [a]	Rec. % [b]
APCYCLINE, India	7	6.920	-1.143	98.857	6.752	-3.543	96.457
SAMACYCLINE, SDI-Iraq	٧	6.854	-2.086	97.914	6.865	-1.929	98.071

[a] Percent Error, [b] Percent recovery of five measurements

3. Conclusions

This study clarifies the feasibility of using potassium permanganate as an oxidizing agent for the spectrophotometry determination of tetracycline hydrochloride in bulk and pharmaceutical preparations (as capsules) by batch and merging-zone flow injection methods. The batch method can be easily carried out because it does not need elaborate handling of the

samples and tedious proceedings for the analysis. Merging-zone flow injection method can be considered as the optimization procedure for the batch method with an uncommon design based on establishing a novel lab-made valve, used in a designed flow injection system. This system is efficient, simple, and inexpensive with ease of repair and cleaning. The two suggested methods were simple, sensitive, and inexpensive, with good linearity and high recovery percent. The flow injection method does not allow the interferences to affect the determination process and is characterized by high sample throughput of 80 samples per hour, with higher sensitivity and higher specific and molar absorption coefficient values than the batch method, which enables this method to be implemented in routine quality control of tetracycline in industrial laboratories.

4. Experimental

4.1. Materials and Methods

A Biochrom Libra S60 double beam spectrophotometer using a matched 1 cm quartz cell was implemented to measure absorbance. The flow injection system consisted of an ismatec peristaltic pump used to transfer the carrier stream solution, and a lab-made valve composed of four secondary valves, each with three exits, was used to load and inject the sample and reagent solutions. (1 mm i. d.) Teflon pipes were used as carrier stream flow lines, loading loops, and mixing coils. A UV–Vis detector (OPTIMA SP300) and chart recorder (KOMPENSOGRAPH C1032) were used to obtain the resulting peaks. All the chemicals were of analytical grade. Tetracycline hydrochloride was kindly provided by the state company for drug industries and medical appliances, Samaraa, Iraq. The pharmaceutical formulations containing tetracycline hydrochloride, such as Apcycline (250 mg) and Samacycline (250 mg), were purchased locally.

The standard solution of 6.33×10^{-3} M potassium permanganate was freshly prepared. In a 100 mL volumetric flask, 0.1 g of $KMnO_4$ has been dissolved in a sufficient volume of distilled water then the volume has been completed with distilled water to the flask mark. Working standard solutions were freshly prepared by appropriate dilution of the standard solution with distilled water. A 100 μg mL^{-1} standard solution of tetracycline hydrochloride was prepared by dissolving 0.01 g of tetracycline hydrochloride in a sufficient volume of distilled water, 0.4 M sodium hydroxide for the batch, flow injection methods, respectively, then completing the volume with the same solvent to the flask mark. Working standard solutions were freshly prepared by appropriate dilution of the standard solution with the specific solvent for each method.

4.2. General Procedure

4. 2. 1. For the Spectrophotometric Batch Method

One milliliter of ١0 μg mL^{-1} tetracycline standard solution was pipetted into a series of 10 mL volumetric flasks, and then 0.2 mL of 18.98×10^{-4} M potassium permanganate solution and 0.25 mL of 0.5 M sodium hydroxide were added. After mixing the reaction mixture, it was left for 3 minutes, and then the absorbance at 610 nm (at room temperature) was measured against a blank solution treated similarly. The calibration plot was built between the absorbance and the concentration in μg mL^{-1}.

4. 2. 2. For Merging-Zone flow Injection Method

Using the flow injection system (**Fig. 5**), aliquots 157.14 μL of tetracycline solutions with different concentrations, 1-25 μg mL^{-1} and 78.57 μL of 6.33×10^{-4} M potassium permanganate solution were loaded together into the sample and reagent loops of the designed valve by means of a syringe. These loaded solutions were brought together in a confluent manner and then transferred towards the reaction coil by pumping the carrier stream of distilled water at a flow rate of 3.6 mL $min.^{-1}$. Subsequently, the sample and reagent zones flowed through the reaction coil in a length of 20 cm, through the reaction coil and reaching the detector, and these zones mixed very well with each other. At the detector, the absorbance will be measured at 610 nm. The absorbance signal was recorded as a peak on a chart recorder. The calibration plot was built between the absorbance peak height and the concentration.

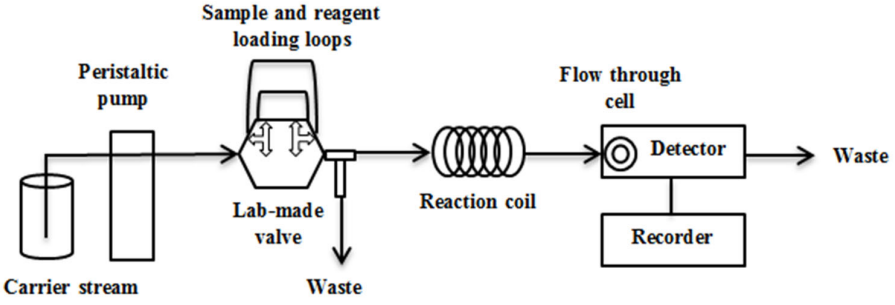

Fig. 5. Schematic diagram of merging-zone flow injection system

4. 2. Recommended Procedure for formulations

The contents of 10 capsules were mixed well and weighed. An accurately weighed quantity equivalent to 0.01 g was dissolved in sufficient distilled water for the batch method or in 0.4 M NaOH for flow injection method. The resultant solution was filtered with Whatman No. 1 filter paper. The filtered solution was brought up to 100 mL with the same solvent to prepare a solution of ١00 µg mL^{-1}. Working standard solutions were prepared by covenant dilution of the standard solution with the same solvent and analysed by the general recommended procedure.

References

1 Hassan, B., and Hadi, H. (**2022**) Development of continuous flow injection analysis method for determination of oxymetazoline and vancomycin hydrochloride in pharmaceutical preparations. *Bull. Chem. Soc. Ethiop.*, 36 (2) 303-313.

2 Santos, A.M., Silva, T.A., Vicentini, F.C., and Fatibello-Filho, O. (**2020**) Flow injection analysis system with electrochemical detection for the simultaneous determination of nanomolar levels of acetaminophen and codeine. *Arabian J. Chem.*, 13 (1) 335-345.

3 Belal, F., Hadi, H., and Jamal, M. (**2019**) Reversed flow-injection method for estimation of chlorpromazine in pharmaceuticals and urine samples using charge-transfer complexation. *Bull. Chem. Soc. Ethiop.*, 33 (1) 11-20.

4 Hameedi, I.T. (**2021**) Determination of tetracycline hydrochloride in pure and pharmaceutical samples via oxidative coupling reaction. *Mater. Today: Proc.*, 42 2953-2958.

5 Khaleel, R.M., and Mohammed, D.H. (**2020**) Spectrophotometric Determination of tetracycline hydrochloride Using 2, 4–dinitrophenyl hydrazine as Coupling Reagent. *J. Phys.: Conf. Ser.*, 1664 (1) 012084.

6 Cao, J., Lai, L., Lai, B., Yao, G., Chen, X., and Song, L. (**2019**) Degradation of tetracycline by peroxymonosulfate activated with zero-valent iron: performance, intermediates, toxicity and mechanism. *Chem. Eng. J.*, 364 45-56.

7 Guerra, P., Kim, M., Shah, A., Alaee, M., and Smyth, S.A. (**2014**) Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. *Sci. Total Environ.*, 473 235-243.

8 Leichtweis, J., Vieira, Y., Welter, N., Silvestri, S., Dotto, G.L., and Carissimi, E. (**2022**) A review of the occurrence, disposal, determination, toxicity and remediation technologies of the tetracycline antibiotic. *Process Saf. Environ. Prot.*, 160 25-40.

9 Yoshizawa, N., Usui, M., Fukuda, A., Asai, T., Higuchi, H., Okamoto, E., Seki, K., Takada, H., and Tamura, Y. (**2020**) Manure compost is a potential source of tetracycline-resistant Escherichia coli and tetracycline resistance genes in Japanese farms. *Antibiotics*, 9 (2) 76.

10 Chen, Y., Su, J.Q., Zhang, J., Li, P., Chen, H., Zhang, B., Gin, K.Y.H., and He, Y. (**2019**) High-throughput profiling of antibiotic resistance gene dynamic in a drinking water river-reservoir system. *Water Res.*, 149 179-189.

11 Gissawong, N., Boonchiangma, S., Mukdasai, S., and Srijaranai, S. (**2019**) Vesicular supramolecular solvent-based microextraction followed by high performance liquid chromatographic analysis of tetracyclines. *Talanta*, 200 203-211.

12 Pang, Y.H., Lv, Z.Y., Sun, J.C., Yang, C., and Shen, X.F. (**2021**) Collaborative compounding of metal–organic frameworks for dispersive solid-phase extraction HPLC–MS/MS determination of tetracyclines in honey. *Food Chem.*, 355 129411.

13 Lanjwani, M.F., Altunay, N., and Tuzen, M. (**2023**) Preparation of fatty acid-based ternary deep eutectic solvents: Application for determination of tetracycline residue in water, honey and milk samples by using vortex-assisted microextraction. *Food Chem.*, 400 134085.

14 Saenjum, C., Pattapong, N., Aunsakol, T., Pattananandecha, T., Apichai, S., Murakami, H., Grupan, K., and Teshima, N. (**2022**) High sensitivity spectrophotometric determination of tetracycline with zirconium chelation by employing simultaneous injection effective mixing analysis (SIEMA): Tetracycline residue in honey. *J. Food Compos. Anal.*, 105 104215.

15 Faria, L.V., Lima, A.P., Araújo, F.M., Lisboa, T.P., Matos, M.A., Munoz, R.A., and Matos, R.C. (**2019**) High-throughput amperometric determination of tetracycline residues in milk and quality control of pharmaceutical formulations: flow-injection versus batch-injection analysis. *Anal. Methods*, 11 (41) 5328-5336.

16 Rodríguez, M.P., Pezza, H.R., and Pezza, L. (**2016**) Simple and clean determination of tetracyclines by flow injection analysis. *Spectrochim. Acta, Part A*, 153 386-392.

17 Jin, L., Qiao, J., Chen, J., Xu, N., and Wu, M. (**2020**) Combination of area controllable sensing surface and bipolar electrode-electrochemiluminescence approach for the detection of tetracycline. *Talanta*, 208 120404.

18 Kuang, G., Wang, C., Song, L., Zhang, G., Yang, Y., and Fu, Y. (**2023**) Novel electrochemiluminescence luminophore based on flower-like binuclear coordination polymer for high-sensitivity detection of tetracycline in food products. *Food Chem.*, 403 134376.

19 Wang, T., Mei, Q., Tao, Z., Wu, H., Zhao, M., Wang, S., and Liu, Y. (**2020**) A smartphone-integrated ratiometric fluorescence sensing platform for visual and quantitative point-of-care testing of tetracycline. *Biosens. Bioelectron.*, 148 111791.

20 Yao, R., Deng, B., Li, Z., Xie, L., Li, J., Tuo, K., Fan, C., and Pu, S. (**2023**) A covalent organic framework rich in lanthanide Eu3+ binding sites for sensitive and selective determination of tetracycline. *Dyes Pigm.*, 213 111159.

21 Weng, X., Huang, J., Ye, H., Xu, H., Cai, D., and Wang, D. (**2022**) A high-performance electrochemical sensor for sensitive detection of tetracycline based on a Zr-UiO-66/MWCNTs/AuNPs composite electrode. *Anal. Methods*, 14 (31) 3000-3010.
22 Xu, H., Zhang, D., Weng, X., Wang, D., and Cai, D. (**2022**) Electrochemically reduced graphene oxide/Cu-MOF/Pt nanoparticles composites as a high-performance sensing platform for sensitive detection of tetracycline. *Microchim. Acta*, 189 (5) 201.
23 Aljeboree, A.M., Abbas, A.S., Abdulrazzak, F.H., Abd Alrazzak, N., and Alkaim, A.F. (**2021**) Role of Selenium dioxide in Spectrophotometric determination of Tetracycline in pure and pharmaceutical formulations. *J. Phys.: Conf. Ser.*, 1999 (1) 012157.
24 J Al-Ashow, R., and S Othman, N. (**2012**) Spectrophotometric determination of tetracycline by coupling with diazotised 4-aminoantipyrine in presence of cetylpyridinium chloride. *Rafidain journal of science*, 23 (3) 72-84.
25 Ali, R.J., Hawezy, H.J.S., and Abdullah, M.S. (**2018**) Spectrophotometric determination of tetracycline hydrochloride through coupling with sulphanilic acid. *Diyala Journal of Medicine*, 15 (2) 15-22.