INTRODUCTION
Pesticides are extensively worldwide used for agriculture and non-agricultural purposes. Pesticides are recalcitrant pollutants that resist the different levels of degradation either chemical, biochemical, or photochemical, because of their chemical characteristics [1]. Bendiocarb (BEN) is chemically known as 2, 2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate (Fig. 1). BEN like other carbamates, this insecticide inhibits the enzyme acetylcholinesterase, necessary for normal transmission of nerve impulses [2,3].

In agriculture, it is used for seed treatment and granular formulations for controlling soil pests and foliage, mainly maize and sugar beet [4]. BEN is also used to eliminate domestic and industrial pests, such as mosquitoes, flies, red ants, and spiders among others [5]. The domestic and industrial use of insecticides, including BEN, has been increased in tropical countries because of the dissemination of diseases whose transmission vectors are insects [6,7]. As for toxicity, toxicological studies of BEN and cases of intoxication in humans have occurred [8]. Many methods have been reported for the spectrophotometric determination of phenols [9,10].

Literature survey revealed that BEN can be determined by several methods such as spectrophotometry [11,12], flow injection analysis [13], high-performance liquid chromatography [14], gas chromatography [15,16], and flow spectrofluorimetry [17].

In the present work, it was possible for the determination of BEN in its formulations and water samples using a simple, accurate, and sensitive flow injection method. A two-channel manifold was employed; the solution of 2,4-Dinitrophenyl hydrazine (DNPH) and NaIO₄ solution streams was combined at Y-junction and then merged with injection sample of hydrolyzed BEN in NaOH (0.2 M) to form red-colored product which measured at 515 nm.

METHODS

Apparatus
A digital double-beam recording spectrophotometer Shimadzu UV-visible 260 (Shimadzu, Kyoto, Japan) was used for absorbance measurements using 1 cm glass cell, in addition to, use of 50 μL internal volume, and 1 cm path length for the FIA measurements. A peristaltic pump (Shenchen, Lab M1, China) was used to transport the carrier solution. An injection valve (Knauer, Germany) was employed to provide appropriate injection volumes. Flexible vinyl tubes (0.5 mm i.d.) were used for the peristaltic pump. Moreover, teflon made reaction coil (R.C) with 0.5 mm (i.d.).

Chemicals
The materials and reagents used in this work with high purity, double-distilled water were used in the preparation of all solutions.

BEN
BEN (500 μg/mL) pesticide (99.0% purity, M wt. 223.23 g/mol) was obtained from Bayer (Frankfurt, Germany). A standard stock solution 500 μg/mL of BEN was prepared by dissolving 0.05 g of pesticides in 4 mL of ethanol and then completed to the mark with distilled water using 100 mL volumetric flask. More dilute solution were prepared by suitable dilution of the stock solution by hydrolyzed in alkaline medium (NaOH, 0.2 M).

DNPH solution (0.01 M)
It was freshly prepared by dissolving 0.19814 g of DNPH (BDH, England, M. Wt 198.14 g/mol) in 4 mL concentrated sulfuric acid and diluted to the mark with distilled water to obtain 0.01 M solution using 100 mL volumetric flask; more dilute solution was prepared using simple dilution with distilled water.

Sodium periodate solution (0.1 M)
A 0.1 M of sodium periodates (BDH, England, 99% purity, mol wt. 213.91 g/mol) was prepared by dissolving 2.1391 g in 5 mL distilled water, and then, the volume made up to mark using 100 mL volumetric more diluted solution was prepared with the same solvent.

Sodium hydroxide (1 M)
It was prepared by dissolving 4 g of sodium hydroxide (BDH, England, M. Wt 40 g/mol) and diluted to mark with distilled water using 100 mL.
volumetric flask to obtain 1 M solution; more diluted solution was prepared with same solvent.

Samples Preparation (500 μg/mL)
The preparation pesticide formulation sample (Ficam 80% W/W, Bayer, Germany) was carried out by weighing 0.0645 g and dissolved in 5 mL ethanol and then adequately diluted with distilled water to mark using 100 ml volumetric flask.

Water sample
About one liter of tap and river water samples was randomly collected from the Tigris river (Baghdad, Iraq). The river water was first filtered off to remove any suspended materials, and all samples were kept in the refrigerator until analyzed. Each sample was spiked with different concentrations of BEN standard solution and subjected to the recommended procedure.

FIA procedure
A two-channel manifold was employed for the FIA spectrophotometric determination of BEN (Fig. 2). The solution of DNPH (0.001) line and NaIO₄ (0.03 M) solution streams were combined at Y-junction, then merged with injection sample (150 μL) of BEN (100 ppm hydrolyzed in 0.2 M of NaOH), and then mixed in the 75 cm R.C at total flow rate of 2 mL/min. The absorbance of the colored product (red color) was measured at 515 nm.

RESULTS AND DISCUSSION
In the series of preliminary attempts to ensure the formation of the colored product between BEN and DNPH, the scanning spectrum of the coupling product carried out in range of 220–900 nm which recorded after obtaining the optimum conditions. The scanning carried out using 20 μg/mL of BEN and 1×10⁻³ M DNPH in the presence of 0.001 M sodium periodate in alkaline medium (using 0.1 M of NaOH). The solutions were mixed in 10 mL volumetric flask, and the red colored product was formed and gave maximum absorption at 515 nm when measured against reagent blank (Fig. 3). The spectra of other individual reaction constituents such as BEN, blank, and colored product solutions were recorded as shown in Fig. 3. Therefore, 515 nm will be used throughout this study as the maximum wavelength.

According to the primary investigation, the aromatic hydrazine group present in DNPH is oxidized by NaIO₄ and the resultant product [18,19] coupled with BEN in para position of its phenolic group (after hydrolysis of BEN in alkaline medium) to form red-colored product in alkaline medium of sodium hydroxide solution. Scheme 1 shows the proposed mechanism which indicated the existence of 1:1 (DNPH:BEN).

Optimization of chemical and physical conditions
According to the previous primary investigation which is adopted as a basis for developing normal flow injection analysis procedure for the determination of BEN. FIA manifold was designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of BEN with DNPH in the presence of NaIO₄ in alkaline medium of sodium hydroxide. Initial studies were directed toward the optimization of the experimental conditions, to find the most suitable parameters for the determination of BEN. The study was carried out by altering one factor and keeping the others constant. Each sample was injected 3 times, and the average absorbance was presented. The preliminary conditions for the proposed FIA procedures are summarized in Table 1.

It was observed that the alkaline medium is very essential for the reaction between BEN and DNPH in the presence of NaIO₄ for developing the colored product; therefore, several alkaline media were examined such NaOH, KOH, NH₄OH, and Na₂CO₃ with using 0.3 M of each base to select the best one that can give more phenolic product of BEN which can affect on the sensitivity of colored product at λ max.

Table 1: Preliminary chemical and physical conditions

| Parameter                      | Value  |
|--------------------------------|--------|
| BEN (μg/mL)                    | 100 ppm|
| Base type                      | NaOH   |
| Base concentration (M)         | 0.3 M  |
| DNPH concentration (M)         | 0.001 M|
| Oxidation agent                | NaIO₄  |
| Oxidation agent concentration (M) | 0.05  |
| Flow rate (mL/min)             | 2.4    |
| Sample volume (mL)             | 150    |
| Reaction coil (cm)             | 50     |

DNPH: 2,4-Dinitrophenyl hydrazine
Fig. 4 shows that NaOH gave the best absorbance compared with other alkaline media; therefore, it was chosen for further use in all subsequent experiments.

The effect of different NaOH concentrations (0.05–0.4 M) was investigated. The results obtained indicated that the absorbance was increased with increasing concentration of NaOH up to 0.2 M, which was selected as optimum concentration in next studies to give the maximum absorbance at 515 nm as shown in Fig. 5.

To select the most suitable oxidizing agent used in this study, different types of oxidizing agent (NaIO₄, KIO₃, Ce⁴⁺, K₂S₂O₇, and K₃[Fe(CN)]₆) were investigated using 0.05 M of each one. It was found that NaIO₄ gave the maximum absorbance more than other oxidizing agent (Fig. 6) and was used in all subsequent experiments.

The influence of sodium periodate as an oxidizing agent plays an important role in the stage of coupling reaction between the hydrolyzed BEN and DNPH. Therefore, the effect of various concentration of sodium periodate was investigated in a range of 0.005–0.07 M. The obtained results (Fig. 7) indicate that the absorbance was increased with the increase in concentration of NaIO₄ up to 0.03 M. However, the absorbance at any concentration beyond this level (0.03 M) led to decrease in absorbance value. Therefore, the 0.03 M of NaIO₄ was selected as the optimum concentration which will be used in the next studies.

The effect of different concentrations of DNPH on the absorption signal was studied according to the recommended procedure through varying the concentration in the range of 0.0001–0.01 M. The results of the DNPH concentration are shown in Fig. 8 which indicated that 0.001M was selected as the optimum concentration due to the negligible blank signal and gave the maximum absorbance. At greater concentrations, the blank signal increased considerably.

The effect of total flow rate on the absorption signal of the colored product was also examined in the range of 0.4–3.6 mL/min. When the flow rate increased, the signal increased up to a flow rate of 2 mL/min (Fig. 9), and there was a decrease for greater flow rates (more than 2 mL/min) because residence time is not enough for the reaction to be completed. A flow rate of 2 mL/min was chosen as a compromise.
among adequate sampling rate and sensitivity. With this flow rate, a sampling rate of 40/h was achieved.

The injection volume was investigated with loops in the 75–250 μL range. The signal increases with increasing sample volume up to 150 μL (Fig. 10a) and remains nearly constant for larger volume. Above this volume (150 μL), a broadening response effect was produced without any increase in the response height. Therefore, a loop of 150 μL was selected next studies to maintain a good sampling rate.

The influence of reaction coil length on the sensitivity was studied in a range of 0 (without reaction coil) to 200 cm. The results showed that increase in the length of reaction coil up to 75 cm leads to increase the sensitivity which is related to the enough residence time for the reaction mixture, whereas a longer reaction coil (more than 75 cm) leads to
decrease sensitivity and also causes response broadening probably due to the dispersion of the sample into the reaction coil. Thus, a 75 cm (Fig. 10b) was selected as the optimum reaction coil length, which will be used in all subsequent experiments.

Calibration curve
Under all optimum conditions mentioned in the previous studies. A linear calibration graph (Fig. 11) was constructed in range of 1–150 μg/mL of BEN standard solution (hydrolyzed with 0.2 M of NaOH). Each measurement was repeated 3 times successively. Analytical values of statistical treatments for the calibration graph are summarized in Table 2 [20].

However, the limit of detection of the proposed method was also calculated [Table 2]. The obtained results have encouraged the proposed method in the estimation of BEN in real samples such as environmental samples to test its applicability and reliability. The developed method in the present work may achieve the requirements of the international standards in terms of the maximum residue limits of BEN insecticide in different types, set by the FAO/WHO [21].

Accuracy and precision [22]
Under the optimum conditions, the accuracy and precision of the proposed method for the determination of BEN were studied using different concentrations of BEN standard solution (500 μg/mL) or pesticide formulation (ficam). Table 3 shows the value percentage of the relative error %, relative recovery %, and percentage relative standard deviation %, respectively, of five replicates of each concentration, which give a good accuracy and precision value for the proposed method.

Application
The proposed method was applied (using standard addition method) for the determination of BEN in pesticide formulations (Ficam 80% w/w, Bayer, Germany) and spiked water samples. According to the preliminary tests, it was found that all the spiked samples of this study do not have any residue of BEN pesticide. Some water samples including river and tap water - were collected in the city of Baghdad, Iraq. Spiked samples were prepared by the addition of some BEN or Ficam, to obtain the required concentration (5, 10, and 25 μg/mL). The results are summarized in Table 4. The proposed procedure is useful in the control of the BEN in pesticide products.

By comparing the proposed method findings with the reported methods [23, 24] in terms of recovery for the water analysis, the F-test and t-test revealed that (Table 5) there is no significant difference between the two methods at 95% confidence interval. The present method gave satisfactory results for the online determination of BEN in water samples without extraction process.

CONCLUSIONS
The obtained results indicated that the proposed method is economical with reasonable precision and accuracy for the determination of BEN in pesticide formulation and water samples at a trace level of BEN (μg/ml) by oxidation coupling reaction with 2,4-dinitrophenylhydrazine in the presence of periodate in alkaline medium. The proposed method is superior to the previously reported spectrophotometric method for the determination of BEN in terms of the simplicity, speed (sample throughput of 40 injection/h), and without requiring a temperature or pH control. Therefore, the method is practical and valuable for routine analysis for the determination of BEN.

AUTHORS’ CONTRIBUTIONS
Sadeem S Abed and Abdul Kareem J Ali conceived of the presented idea. Malik H. A. Alamri developed the experimental procedure and performed the computation. All the authors discussed the results and contributed to the final manuscript.
Table 5: The comparison of the proposed method with classical methods using t-test and F-statistical tests

| Sample          | Rec % | Proposed method | Classical method |
|-----------------|-------|-----------------|------------------|
| Pure BEN        | 100.51| 95.90           |                  |
| Tap water       | 97.60 | 97.28           |                  |
| River water     | 97.276| 96.420          |                  |
| t (2.776)       | +1.75 | -               |                  |
| F (19.0)        | 6.53  | -               |                  |

REC %: Recovery %, BEN: Bendiocarb

CONFLICTS OF INTEREST

Authors have no conflicts of interest.

REFERENCES

1. Christos AD, Ilias GE. Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 2011;8:1402-19.
2. Wood A. Compendium of Pesticide Common Names. London: Index, Bendiocarb; 2016.
3. Eckert S, Eyer P, Worek F. Reversible inhibition of acetylcholinesterase by carbamates or Huperzine A increases residual activity of the enzyme upon soman challenge. Toxicology 2007;233:180-6.
4. Worthing CR. The Pesticide Manual: A World Compendium. 7th ed. Lavenham, Suffolk: Lavenham Press Ltd.; 1983.
5. Lozano-Fuentes S, Hayden MH, Welsh-Rodriguez C, Ochoa-Martinez C, Tapia-Santos B, Kobylinski KC, et al. The dengue virus mosquito vector Aedes aegypti at high elevation in Mexico. Am J Trop Med Hyg 2012;87:902-9.
6. Cetron M. Revision to CDC’S zika travel notices: Minimal likelihood for mosquito-borne zika virus transmission at elevations above 2,000 meters. Morb Mortal Wkly Rep 2016;65:267-268.
7. Dhimal M, Gautam I, Joshi HD, O’Hara RB, Harens B, Kuch U. Risk factors for the presence of chikungunya and dengue vectors (Aedes aegypti and Aedes albopictus), their altitudinal distribution and climatic determinants of their abundance in central Nepal. PLoS Negl Trop Dis 2015;9:e0003545.
8. Petrovova E, Purzych H, Mazensky D, Luptakova L, Torma N, Sopolina I, et al. Morphometric alterations, steatosis, fibrosis and active caspase-3 detection in carabamate bendiocarb treated rabbit liver. Environ Toxicol 2015;30:212-22.
9. Israa MI, Sadeem SA. Flow injection spectrophotometric determination of narigenin in supplements using solid-phase reactor containing immobilized manganese dioxide. Asian J Pharm Clin Res 2018;11:473-8.
10. Patel PA, Dole MN, Shedpure PS, Sawan SD. spectrophotometric simultaneous estimation of salbutamol and ambroxol in bulk and formulation. Asian J Pharm Clin Res 2011;4:42-5.
11. Saadyah AD, Jehan SH, Kareem DK. Spectrophotometric determination of bendiocarb after synthesis a new colored compound with a para-amino phenol in environment water sample. Int J Chem Sci 2015;13:415-31.
12. Zuhair A, Suher SA. Micelle-mediating extraction combined with visible spectrophotometry for the determination of ultra trace amounts of bendiocarb insecticide in various matrices after oxidative coupling with O-Toluidine. Int J Res J Pure Appl Chem 2016;10:1-16.
13. Alvarez-Rodriguez L, Esteve-Romero J, Escrig-Tena I, Alvarez-Coque MC. Flow-injection spectrophotometric determination of phenolic drugs and carbamate pesticides by coupling with diazotized 2,4,6-trimethylaniline. J AOAC Int 1999;82:937-47.
14. Menezes M, Félix G. On line extraction and separation of bendiocarb, methomyl, methyl parathion, and pentachlorophenol pesticides from raw milk. J Liq Chromatogr Relat Technol 1998;21:2863-71.
15. Szeto S, Wilkinson A, Brown M. A gas chromatographic method for the determination of bendiocarb in soil and corn: Application to the analysis of residues in corn. J Agri Food Chem 1984;32:78-80.
16. Farber H, Scholer F. Gas chromatographic determination of carbamate pesticides after flash-heater methylation with trimethylsulfonium hydroxide. J Agri Food Chem 1993;41:217-20.
17. Pacioni N, Veglia A. Determination of poorly fluorescent carbamate pesticides in water, bendiocarb and promecarb, using cyclodextrin nanocavities and related media. Anal Chim Acta 2007;583:63-71.
18. Al-Abachi MQ, Sadeem S. Flow injection-spectrophotometric determination of salbutamol sulphate and pyridoxine hydrochloride using 2,4-dinitrophenylhydrazine. Iraqi J Sci 2013;54:6-16.
19. Al-Abachi MQ, Sadeem S. Flow injection-spectrophotometric determination of phenylephrine hydrochloride and amoxicillin trihydrate in pharmaceutical preparations. J Al-Nahrain Univ 2013;16:42-52.
20. Miller JN. Statistics and Chemometrics for Analytical Chemistry. 6th ed. United Kingdom: Pearson Education Limited; 2010.
21. Codex Alimentarius Commission. Pesticides Residue in Food. 2nd ed. Joint FAO/WHO Food Standard Programme of United Nations;. p. 127.
22. Harris DC. Quantitative Chemical Analysis. 7th ed. California: Michelson Laboratory, China Lake; 2007.
23. Kumar KS, Suvardhan A, Redha D, Kiran K, Jayaraj B, Janardhanam K, et al. Development of simple and sensitive spectrophotometric method for the determination of bendiocarb in its formulations and environmental samples. Environ Monit Assess 2007;127:67-72.
24. Leo ML, Hamir SR. Handbook of Pesticides: Methods of Pesticide Residues Analysis. New York: Taylor and Francis Group; 2010.