Determination of Bendiocarb Insecticide by Reverse Flow Injection Analysis Using a Solid-Phase Reactor Containing Micro PbO_2, Nano PbO_2 and Grafted SiO_2 - PbO_2 Immobilized

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Summary: Bendiocarb **(**BEN) is an acutely toxic carbamate insecticide which used in public places and agriculture, it is also effective against a wide range of nuisance and disease vector insects. A new rapid and sensitive reverse flow injection spectrophotometric procedure coupled with on-line solid-phase reactor is designed in this article for the determination of BEN in its insecticidal formulations and water samples, by using three different solid-phase reactors containing bulk PbO_2 (B-SPR), PbO_2 nanoparticles (N-SPR) and grafted nanoparticles of SiO_2-PbO_2 (G-SPR) immobilized on cellulose acetate matrix (CA). This method of oxidative coupling is based on alkaline hydrolysis of the BEN pesticide, and then coupled with N,N dimethyl-p-phenylenediamine sulphate **(**DMPD**)** to give a blue color product which measured at λ_{max} 675 nm. It worth to mentioned that under optimal conditions, Beer's law is obeyed in the range of 1-175 μg mL^{-1} for B-SPR and 0.25-70 μg mL^{-1} of BEN for both N-SPR and G-SPR respectively within limit of detection (LOD) of 0.931, 0.234 and 0.210 μg mL^{-1} for B-SPR N-SPR and G-SPR respectively. The surface methodology of the solid phase was also investigated by using atomic force microscopy.

Keywords: Bendiocarb; Flow injection; Solid phase reactor and Immobilized nano PbO_2 – SiO_2.

Introduction

Pesticides are substances or a mixture of chemical or biological origin, used by human society to mitigate or repel pests and other organisms that affect food production or human health. Pesticides usually act by disrupting some component of the pest's life processes to kill or inactivate it. Although pesticides have benefits, some also have drawbacks, such as potential toxicity to humans and other species [1-3]. Carbamates are recently was developed widely in pesticides. Carbamate insecticides structurally are derived from carbamic acid and substituted phenols. Carbamates toxicity is similar toxic action to that of organophosphates [4,5]. Moreover, the action of Carbamate insecticides in killing insects is by reversibly inactivating the acetylcholinesterase enzyme. In same manner, the organophosphate pesticides also inhibit this enzyme, although irreversibly, and cause a more severe form of cholinergic poisoning [6-8]. For example, Bendiocarb (BEN) is a carbamate insecticide and its chemically known according to IUAPAC system as 2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate. Physical properties of BEN (Fig. 1) are a white to light brown powder with low solubility in highly polar solvents such as water, but it is very soluble in various organic solvents of intermediate polarity. Therefore, BEN is rapidly hydrolyzed under moderately alkaline conditions, but slowly hydrolysed under moderately acidic conditions [8, 9]. It is good to know that BEN is one of 12 insecticides recommended by the World Health Organization to control malaria disease [10]. It is also not considered being carcinogenic, but in same time is acutely toxic. Many formulations of BEN are registered for general use and sold under the trade names such as "Ficam" and "Turcam". In addition to that, BEN is active against many kinds of pests [11, 12].

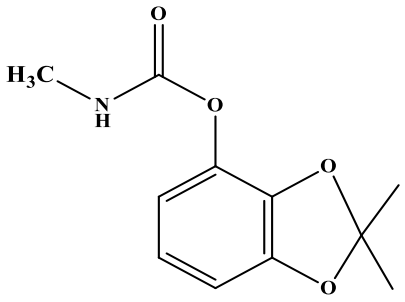

Fig. 1: The chemical structure of bendiocarb ($C_{11}H1_3NO_4$).

Solid-phase reactors (SPR) methods have become one of the most interesting trends in continuous-flow methodologies in spite of those cited shortcomings, mainly due to the advantages that are not available in soluble form reagents. Using of SPR in FIA has been reviewed and also has many

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applications to pharmaceutical analyses [13-16]. Another method are Nanoparticles which becoming increasingly important in many areas including catalysis, biomedical applications and others, due to their high surface area and nanoparticles (NPs) size. NPs has unique physical and chemical properties corresponding to bulk or microcrystalline materials make these materials superior in different forms [17, 18]. Recent advances in controlling the size and shape of NPs have opened the possibility to optimize the particle geometry for enhanced catalytic activity, providing the optimum size and surface properties for specific applications [19]. According to literature survey that BEN can be determined by several methods such as UV-Vis spectrophotometry [20-22], gas chromatography (GC) [23] high performance liquid chromatography (HPLC) [24] chromatography-mass spectrometry [25] and flow injection analysis [26].

In this study, rFI spectrophotometry method coupled with solid phase reactor was proposed for the determination of BEN. The method was successfully applied for determination of BEN in its insecticidal formulations and spiked water samples with good accuracy, precision. And without detectable interference by standard-addition procedure and the methods were to be simple, accurate and easy to apply to routine analysis.

Experimental

Bendiocarb

(BEN, 500 μg mL^{-1}) pesticide (99.0% purity, M wt. 223.23 g. mol^{-1}) was obtained from Bayer (Frankfurt, Germany). A standard stock solution 500 μg mL^{-1} 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 solutions were prepared by suitable dilution of the stock solution by hydrolyzed in alkaline medium (0.2 M, NH_4OH).

N,N dimethyl-p-phenylenediamine sulphate (DMPD) (0.01M)

DMPD reagent (209.12 g. mol^{-1}, BDH, UK) was prepared freshly by dissolving 0.10456 g of DMPD in distilled water and the volume was completed with same solvent to 50 mL to obtain 0.01 M solution. The solution was kept in a dark bottle.

Ammonium hydroxide solutions (1M)

These solutions were prepared by appropriate dilution of the standard solution (Fluka, Switzerland, M.Wt. 35 g mol^{-1}) of ammonium hydroxide (1M) with distilled water in 100 mL volumetric flask.

Samples Preparation (500 μg mL^{-1})

Preparation of pesticide formulation sample (Ficam 80 % W/W, Bayer , Germany) was carried out by weighting 0.0645 g, and dissolved in 5 mL ethanol, and adequately diluted with distilled water to mark of 100 ml volumetric flask.

Water Sample

One liter of each 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 test. Each sample was spiked with different concentration of BEN standard solution and subjected to the recommended procedure.

Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV/VIS 260 digital double beam recording spectrophotometer (Japan). A flow cell with 50 μL internal volume and 1 cm path length was used for the absorbance measurements. A one-channel manifold was employed for the rFI spectrophotometric determination of BEN. A peristaltic pump (Shennchen, Lab M1, China) was used to transport the carrier solution. An injection valve (Knauer, Germany) was employed to provide appropriate injection volumes of the standard solutions and samples. Flexible vinyl tubes of 0.5 mm internal diameter (i.d.) was used for the peristaltic pump. Moreover, teflon made reaction coil (R.C) with 0.5 mm (i.d.) was utilized.

Atomic Force Microscope

The surface morphology of PbO_2 nanoparticles was estimated using AFM (AA3000 Angstrom advanced Inc).

Preparation of solid *-phase reactor containing immobilized bulk PbO_2 (B-SPR)*

The immobilization of PbO_2(s) was similar to that previously reported [27]. Cellulose acetate (CA) (0.5g) was dissolved completely in 3 mL acetone and 0.5 mL of dimethyl formamide with continuous stirring. This was followed by addition of 4 g of PbO_2 powder to the mixture. The mixture

was homogenized by manual mixing until increased in viscosity was observed.

Ten minutes later the homogenized mixture was washed with water and rigid polyester was obtained. After air-drying, the polyester containing the immobilized PbO_2 was crashed into desirable sizes. The suitable particle size subsequently being selected (0.15 – 1.18 μM) by sieving on known mesh sieves (50mm × 200mm, Retsch GmbH & Co.KG, Germany). Finally, the selected particles were washed, dried at room temperature then sieved again and stored. The SPR was prepared by packing the particles into glass tubes of different lengths with 2 mm i.d. Small pieces of sponge were inserted at the ends of the tubes to hold the particles in place.

Preparation of solid -phase reactor containing nanoparticles of PbO_2 (N SPR)

The nanoparticles were prepared using sonication method. The PbO_2 particles were dispersed and cracked using ultra-sonication technique, thereby increasing the contribution of their surface area. Such an ultrasonic treatment resulted in an enhancement in the oxidation activity has been observed. Ultrasonic waves also have been found to inhibit the formation of PbO_2 particles larger than 150 nm [28]. The N-SPR was prepared as described steps for preparation of B-SPR as mentioned above but by using NPs of PbO_2.

Preparation of solid -phase reactor containing nanoparticles of PbO_2 grafted with Nanoparticles of SiO_2 filler (G-SPR)

According to previous step for preparation of PbO_2 NPs, the grafted method was carried out by adding NPs of SiO_2 (16 nm as particle size) as a filler substance. Cellulose acetate (CA) (0.5g) was dissolved completely in 3 mL acetone and 0.5 mL of dimethyl formamide with continuous stirring. Then was followed by adding SiO_2 NPs to the solution, this was followed by adding 4 g of PbO_2 NPs. The same procedure steps mentioned above for preparation of B-SPR were used to prepare G-SPR.

Flow injection procedure

A schematic diagram of the rFI manifold contained one channel is shown in Fig. 2. The reagent (DMPD, 0.005 M) was injected into stream of hydrolyzed BEN in 0.2 M of NH_4OH (50, 10, and 10 μg mL^{-1} of BEN for B-SPR, N-SPR and G-SPR respectively), at a flow rate of 1.8 ml min^{-1}. The inserted mixture of hydrolyzed BEN and DMPD when flowing through the solid-phase reactor (8 cm for B-SPR and 6 cm for each of N-SPR and G-SPR with 0.2 mm i.d.) packed with 0.22 g for B-SPR and 0.208 g for both N-SPR and G-SPR. The oxidant agent led to oxidizing of DMPD (entrapped PbO_2 by a polymeric cellulose acetate; ratio of PbO_2: resin, 4: 0.5 for B-SPR and 4: 0.25 g for both N-SPR and G-SPR; with particle size1 mm) then the solution pass thought the 25 cm reaction coil. The absorbance of the colored product (blue color) was monitored at 675 nm.

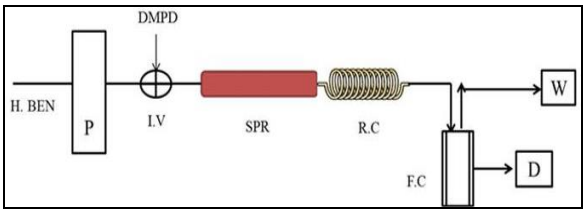


Fig. 2: Schematic diagram of rFI manifold; P, Peristaltic pump; I.V, Injection valve; R.C , Reaction Coil; F.C, Flow Cell; D, Detector; W, waste; H.BEN, hydrolyzed BEN in NH_4OH solution and SPR, solid phase reactor.

Results and Discussion

The preliminary investigation of the proposed reaction was carried out in a 10 mL volumetric flask. A flask containing 25 μg mL^{-1} of BEN, a volume of 0.5 mL (0.005 M) of DMPD, and an amount (0.1 g) of PbO_2 immobilized on CA were added and followed with addition of 0.5 mL (0.4M) of ammonium hydroxide. The flask was swirled immediately and made up to the volume with water and then filtered. The maximum absorption of the product was recorded at 675 nm. Fig. 3 shows the absorbance spectrum of BEN, Hydrolyzed BEN and blank in addition to colored product spectrum which measured against reagent blank.

The proposed reaction mechanism for the coupling of BEN and DMPD postulated as shown in Scheme-1. DMPD is oxidized by PbO_2 losing two electrons and a proton to yield reactive diethylbenzoquinone-diimine which couples with BEN by electrophilic attack at their nucleophilic site, under slightly alkaline conditions, preferably at para-position of hydrolyzed BEN [29, 30] to give a blue colored product at 675 nm.

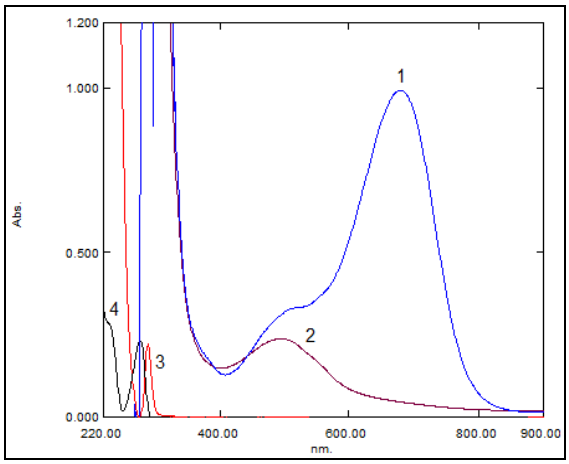
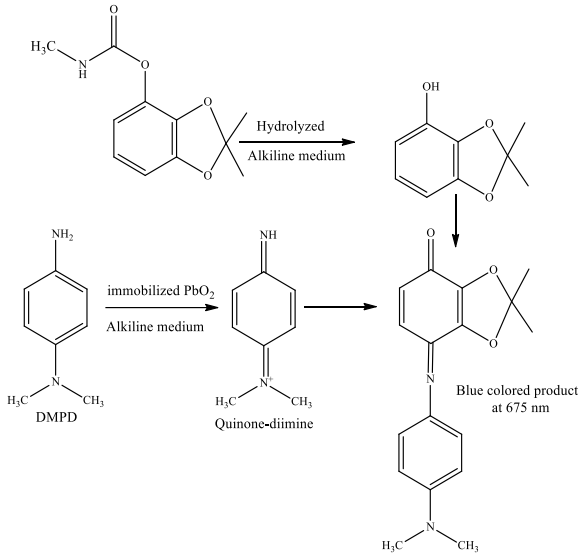


Fig. 3: Absorbance spectrum of, 1; colored product, 2; reagent blank, 3; hydrolyzed BEN and 4; BEN.



Scheme-1: The proposed reaction mechanism between BEN and DMPD in alkaline medium with presence of immobilized PbO_2.

Preparation and characterization of PbO_2 nanoparticles

The pulsed sonication technique has been applied to the synthesis of PbO_2 NPs. This method is generic in nature and is intended to provide relevant guidelines for the preparation of nanoparticles dispersions in liquid media by the application of ultrasonic energy (a process referred to here as sonication). Ultrasonic waves are generated in a liquid suspension either by immersing an ultrasound probe or "horn" into the suspension. The study for preparation of PbO_2 nanoparticles was carried out using, 1g of PbO_2 and 50 ml of acetone as dispersion medium using 75 ml beaker in addition to use of constant sonication energy at room temperature. After period of sonication, the solution obtained was left to stand for a period. Subsequently, the precipitate was collected and dried at room temperature. Finally, PbO_2 nanoparticles of different sizes were obtained. The obtained results indicated sonication method could be used as a confident and controllable method for preparation of lead dioxide NPs. AFM was used to characterize the formed PbO_2 NPs. The data obtained from AFM topography imaging were helpful to obtain information on the preparation mechanism of PbO_2 NPs. 50 µL of the sonicated PbO_2 were put on a cleaned glass slid, and then incubated for period depending on the density of particles required, and then the sold residual was used for AFM measurements. This general method was followed for all sonicated samples. In Fig. 4, AFM images recorded on the samples with shortest and longest sonication times (15-90 min). For the 15 min sonication time, an average size of 103.5 nm was determined. On the other hand, for the sample obtained with longer sonication times, the size distribution appears smaller. The size distribution analysis confirmed that the nanoparticles size decrease with the increasing sonication time. Measurements were performed in ambient conditions. Generally the size of colloidal nanoparticles under investigation were 103.5, 102, 64.8, and 60.7 nm for 15, 30, 60, 90 min respectively therefor, 90 min was chosen as the sonication time for producing PbO_2 NPs which will be used in all subsequent experiments for preparation of N-SPR and G-SPR.

Optimization of the experimental conditions

The effects of chemical and physical parameters on the intensity of the colored product were investigated for the rFI method. The study of optimum conditions was carried out by altering one factor and keeping the others constant. The injection of three times and the average absorbance was presented. The performance of SPR can be affected by several parameters therefore some factors were evaluated. Before the first injection, all tested of solid-phase reactors were conditioned by washing the reactor with distilled water for ten minutes, and then passing the carrier solution for 10 min for the best compaction of the particles in the column. The preliminary conditions for the proposed rFI procedure using three kinds of reactors were tabulated in Table-1.

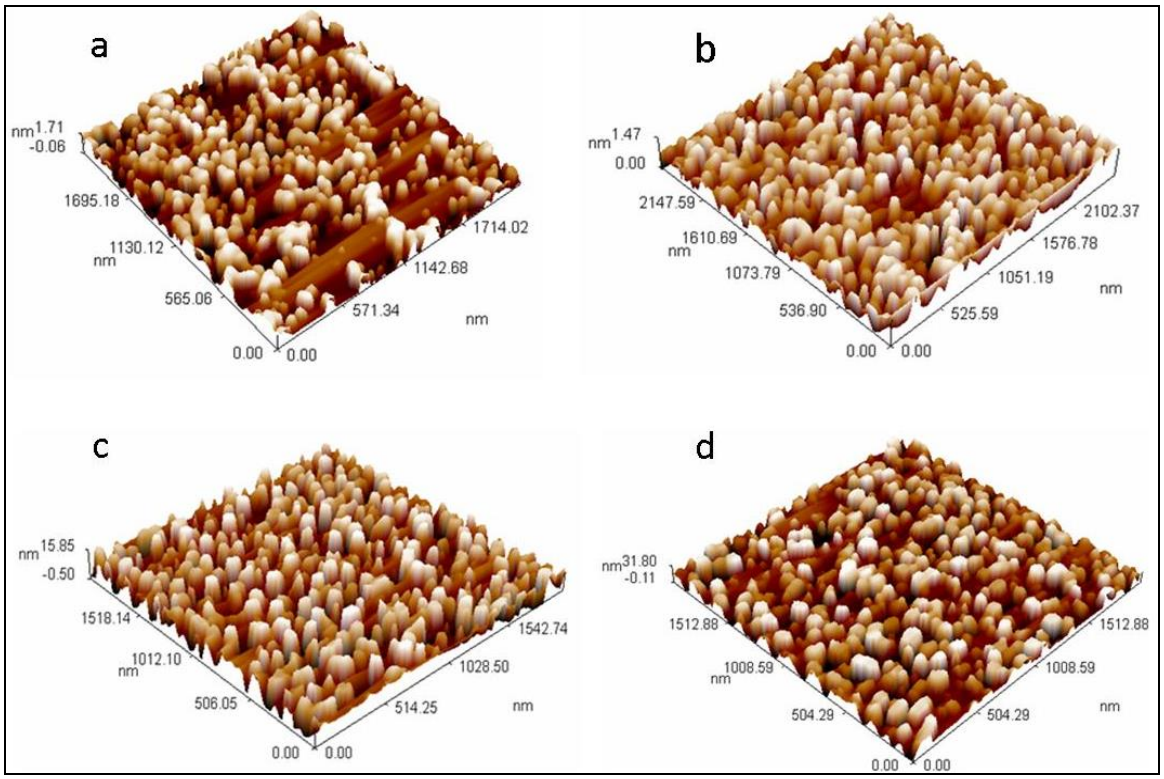


Fig. 4: 3D AFM images of PbO_2 nanoparticles at different periods; a:15, b: 30, c:60 and d: 90 min.

Table-1: The experimental conditions for the proposed rFIA procedure.

Parameter	Value		
	B-SPR	N-SPR	G-SPR
Concentration of BEN (µg mL^{-1})	50	10	10
Concentration of DMPD (M)	0.005	0.005	0.005
Concentration of NH_4OH (M)	0.5	0.5	0.5
Total flow rate ($mL.min^{-1}$)	1.2	1.2	1.2
Reaction coil 1 (cm)	25	25	25
Sample volume (µL)	100	100	100
Ratio of PbO_2 : CA:SiO_2 (w:w, g)	4:0.5:0	4:0.5:0	4:0.5:0.05
Reactor length (cm)	8	8	8
Particles size (mm)	1.18	1.18	1.18
Particles weight (g)	0.15	0.15	0.15

The proportion of PbO_2(s) immobilized (PbO_2: CA, w: w, g) in polyester (CA) has an important effect on reactivity of the oxidant column. Different weight ratios of immobilized PbO_2 in polyester resin were used in the preparation of the solid-phase materials for each reactor (B-SPR, N-SPR and G-SPR). It was found that the ratio of 4:0.5 for B-SPR and 4:0.25 for both N-SPR and G-SPR respectively, which provided highest absorbance and good reproducibility for mentioned reactors as shown in Fig. 5. And it will be used in all subsequent experiments.

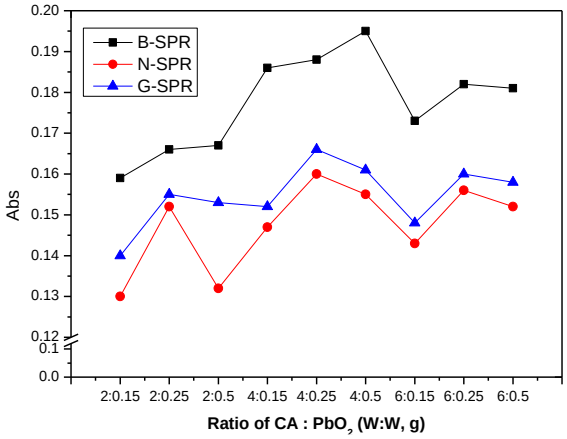


Fig. 5: The effect of CA / PbO_2 ratio against absorbance for B-SPR, N-SPR and G-SPR.

The role of particles size on absorption was investigated and performed to identify a better balance between the magnitude and precision of analytical signals and the stability of baseline. The effect of particle size was studied in different sizes (0.15 -1.18 mm mesh, but the SPR had the same packing weight (0.15 g of immobilized PbO_2). The desirable size particles were collected by passing the particles through mesh sieves of known sizes. The results (Fig. 6) show that the absorbance increased with increasing the

particles size up to 1 mm for B-SPR, N-SPR and G-SPR because the particles size smaller than 1 mm leads to a high flow resistance causing low absorbance. As a compromise, of sensitivity and reproducibility were obtained, the 1 mm particle size was chosen and used in all subsequent experiments.

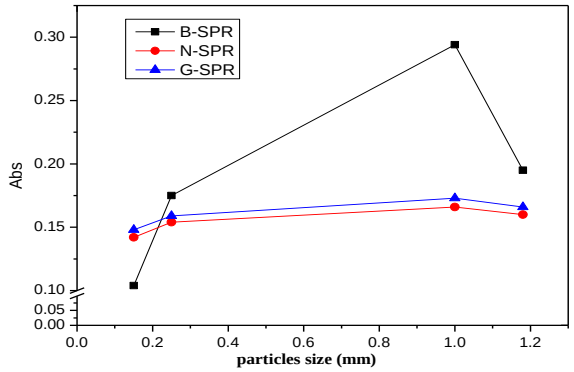


Fig. 6: The solid-phase particles size on absorbance for B-SPR, N-SPR and G-SPR.

The effect of reactor length (4 -12 cm) on the analytical response was studied. It was found that the highest analytical signal was obtained when an 8 cm reactor length was employed for B-SPR, where 6 cm was selected for both N-SPR and G-SPR respectively, as shown in Fig. 7. The 4 cm reactor length gave a lower absorbance owing to the short residence time. On the other hand the use of 12 cm reactor length lead to decrease the absorbance less than those obtained with length of 6 and 8 cm reactors probably due to higher dispersion of sample zone. Therefore, the length of 8 cm for B-SPR and 6 cm for both N-SPR and G-SPR respectively were chosen and used in all subsequent experiments.

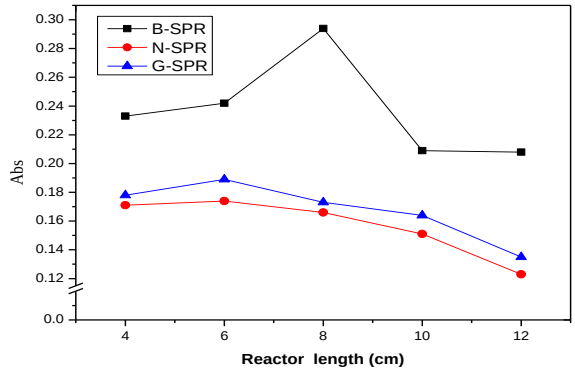


Fig. 7: The solid-phase reactor length effect for B-SPR, N-SPR and G-SPR.

The effect of the solid-phase particles weight (Degree of packing) on the absorbance was optimized using different amounts of the PbO_2 immobilized on CA in the range of 0.050 – 0.24 g, with using the same particles size of 1 mm and the same reactor length for each reactor. The obtained results (Fig. 8) indicate that the absorbance increased with increasing the weight of PbO_2 particles (immobilized on CA) up to 0.22 for B-SPR and 0.208 g for both N-SPR and G-SPR, above this weight the absorbance decreased since there was a resistance to sweeping the reaction plug through the reactor, because of the strong packing of solid-phase material causes an increase in the resistance against the flow of solution. Therefore, a 0.220 for B-SPR and 0.208 g for both N-SPR and G-SPR, particle weight were chosen and used for further subsequent experiments.

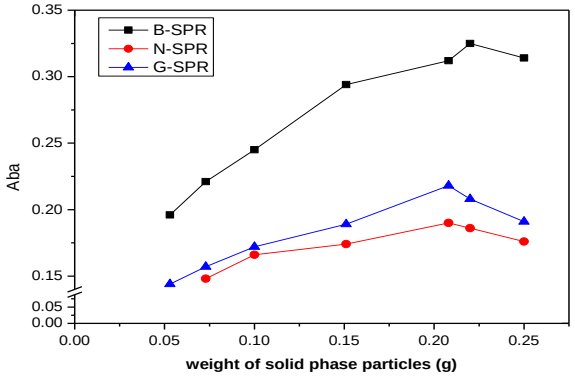


Fig. 8: The solid-phase particles weight effect on the absorbance for B-SPR, N-SPR and G-SPR.

The effects of nano-SiO_2 (16 nm) fillers amount on absorbance have been investigated with a view to its use as a support surface after interaction with CA in presence of PbO_2 NPs. These experimentally measured properties have also been compared with those obtained results from B-SPR and N-SPR. Both the experimental and theoretical trends of these properties with added filler contents correlate very well. The effects of filler composition of solid phase resin composites were assessed using series of fillers amount 0.015 to 0.1 g. According to obtained results (Fig. 9) of grafted resin composites showed significantly higher absorbance for colored product at use of nano-SiO_2, further increase in absorbance is observed up to 0.05g and there is no significant at use more than 0.05 g. Therefore 0.05 g of SiO_2 will be used in all subsequent experiments for the G-SPR.

The hydrolysis of BEN can be conducted in basic medium; Therefore, several alkaline media was chosen (NaOH, KOH, NH_4OH, and Na_2CO_3 at 0.4 M) to select the best base that give the phenolic product of BEN hydrolysis. rFI manifold in Fig. 2 was selected to produce the best conditions. The obtained results

showed that 0.4 M of NH_4OH gave the best absorbance for B-SPR, N-SPR and G-SPR compared with other alkaline media. Therefore, NH_4OH was chosen as the best base for hydrolysis of BEN which gave suitable sensitivity.

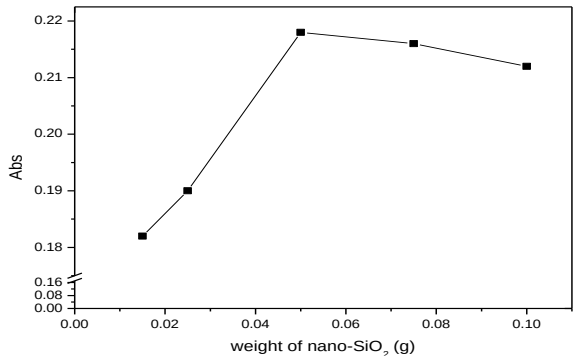


Fig. 9: The nano-SiO_2 weight effect on the absorbance for G-SPR.

It was observed that the alkaline medium is very essential for the reaction between DMPD and BEN for developing the blue colored product; therefore the effect of various concentrations of NH_4OH (0.02 to 0.6 M) was investigated. The obtained results indicated that the absorbance was increased with increasing the concentration of NH_4OH up to 0.2 M, which was selected as optimum concentration in next studies for B-SPR, N-SPR and G-SPR.

The influence of DMPD concentration on the absorbance was evaluated in the range from 0.0005 to 0.009 M which injected through carrier stream (hydrolyzed BEN by 0.2 M NH_4OH). It was observed that the response increased with the increase in DMPD concentration up to 0.005 M for the studied reactors, after this concentration the absorbance was decreased. Therefore, a 0.005 M of DMPD solution was chosen for further experiments.

Initially, the most appropriate flow rates were investigated, since the lifespan of the solid-phase reactor is directly related to the solution flowing through it. The effect of carrier stream flow rate on the absorbance (hydrolyzed BEN with 0.2 M of NH_4OH) was investigated from 0.6 to 2.17 $mL.min^{-1}$. The lower flow rate was found to give lower absorbance signals probably due to higher dispersion of the sample zone. Whereas at higher flow rates more than 1.8 $mL.min^{-1}$, the absorbance signals decreased due the lower contact between the sample zone and the immobilized oxidizer. In addition to, shorter residence time of sample zone and an excessive hydrodynamic pressure occurred. Therefore, 1.8 $mL.min^{-1}$ was selected as best flow rate taking into account the magnitude of the analytical signal, stability of the baseline, and increasing lifespan of the studied solid-phase reactors.

The effect of varying sample loop volume from 75 to 200 µL on the analytical signal was evaluated by injection of the optimum concentration of DMPD passed through the column with immobilized oxidizer. The increase of the sample volume resulted in an increase of absorbance up to 100 µL whereas the absorbance staying practically constant for higher volumes. This behavior can indicate that the quantity of DMPD in 100 µL, for these conditions, to be the maximum necessary for the colored product formation. Therefore, a sample volume of 100 µL was selected for B-SPR, N-SPR and G-SPR because of better engagement between sensibility and analytical frequency. Additionally, increasing lifespan of the solid-phase reactor and decreasing the consumption of chemicals.

The influence of the mixing coil length was investigated in range of 0 - 100 cm the reaction coil placed after SPR then joined to detector as shown in Fig. 2. It was observed that the absorbance increase continuously with an increase in the coil length up to 25 cm while at higher lengths led to decrease in the analytical signal, this fact can be explained by the higher effect of the sample zone dispersion. Therefore the optimum length of reaction coil was 25 cm for B-SPR, N-SPR and G-SPR respectively, which will be used in all subsequent experiments.

Solid-phase reactor life-time

Under the optimum conditions, the sampling frequency was evaluated by recording the time from the sample injection to the maximum absorbance (40 sec). The column lifetime, in terms of its quantitatively ability to give highly absorbance of colored product. It was found that the solid phase reactors (B-SPR, N-SPR and G-SPR) could successfully be used for loading desirable number of reagent injections of 35, 42 and 48 injections with RSD% of 4.37, 3.95 and 3.79 for B-SPR, N-SPR and G-SPR respectively, and it was found that the reproducibility (RSD $\leq$ 5) of reactors was good as well as life time. In addition to the reactors material that was prepared gives high stability more than one month.

Calibration curve

Under all optimum conditions a series of solutions containing 1-175 µg mL^{-1} for B-SPR and 0.25-70 µg mL^{-1} of BEN for both N-SPR and G-SPR respectively, the required concentrations were prepared by appropriate dilution of the stock solution (500 µg mL^{-1}) to provide final required concentration, which hydrolyzed with 0.2 M of NH_4OH. Each measurement was repeated three times successively. The analytical

values of statistical treatments for the calibration graph (Fig. 10) are summarized in Table -2.

However, the LOD of the proposed method was also calculated (Table - 2) for each reactor. 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 present work may achieve the requirements of the international standards in terms of the maximum residue limits (MRL) of BEN insecticide in different types, set by FAO/WHO [31].

The accuracy and precision of the proposed method for the determination of BEN were studied using different concentrations of BEN. Table -3 shows the value percentage of the relative error (E %), relative recovery (Rec. %), and percentage relative standard deviation (RSD %) respectively, for two replicates of each concentration using B-SPR, N-SPR or G-SPR. It can be seen from obtained results that all the used reactors successful to give a good accuracy and precision value for determination of BEN depending on the RSD% value (Table-3).

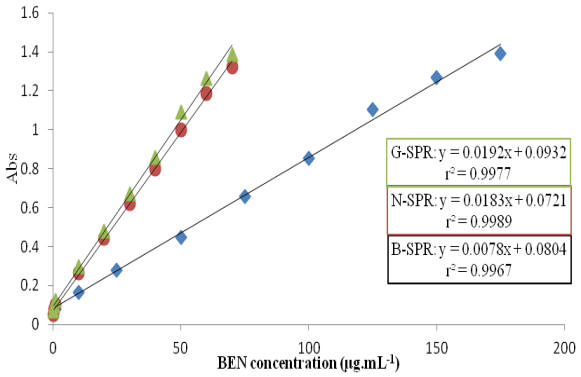


Fig. 10: The calibration curve for the determination of BEN.

Table-2: Analytical values of statistical treatments for the calibration graph using B-SPR, N-SPR or G-SPR, for determination of BEN.

G-SPR	N-SPR	B-SPR	Parameter
Value			
Y= 0.0192x +0.0932	Y= 0.0183x + 0.0721	Y= 0.0078x + 0.0804	Regression equation
0.9988	0.9995	0.9983	Correlation coefficient, r
0.9977	0.9989	0.9967	Linearity percentage, r^2 %
0.25-70	0.25-70	1 – 175	Linear range (µg.mL^{-1})
0.0255	0.0165	0.0298	Standard deviation of the residuals, $S_{y/x}$
0.00032	0.00021	0.00016	Standard deviation of the slope, S_b
0.0122	0.0079	0.0166	Standard deviation of the intercept, S_a
0.21	0.234	0.931	Limit of Detection (LOD) (µg.mL^{-1})
0.625	0.710	2.821	Limit of Quantitation (LOQ) (µg.mL^{-1})

Application for determination of BEN in water samples

The proposed method of rFI coupled with SPR was applied for the quantitative determination of BEN in pesticide formulations (Ficam 80 % w/w, Bayer, Germany) and spiked water samples using standard addition method. According to the preliminary tests, it was found that all the spiked samples of this study do not have any residue of BEN insecticide. Some water samples including river, and tap water — were collected in the city of Baghdad (Iraq). Spiked samples were prepared by addition of some BEN or Ficam, to obtain the required concentration. The obtained results are summarized in Table - 4.

Table-3: The accuracy and precision values; of the proposed method for the determination of BEN in standard solution.

RSD% n=6	RE%	REC%	BEN concentration (µg mL^{-1})	
			Found *	Present
B-SPR				
1.14	- 2.672	97.32	24.33	25
1.23	-3.71	96.29	96.29	100
N-SPR				
0.95	-2.9	97.10	19.42	20
1.08	- 2.375	97.625	39.05	40
G-SPR				
0.66	-2.40	97.60	19.52	20
0.83	-2.25	97.75	39.10	40

* Average of two determinations

Table-4: Application of the proposed method for determination of BEN in pesticide.

RSD% n=5	RE%	REC%	BEN concentration (µg mL^{-1})		
			Found *	Present	
B-SPR					
1.30	-3.40	96.60	4.830	5	Tap water
1.70	-2.45	97.55	19.51	20	
1.56	-2.20	97.80	4.89	5	River water
1.20	-1.90	98.10	19.62	20	
N-SPR					
1.15	-1.80	98.20	4.91	5	Tap water
1.24	-2.15	97.85	19.57	20	
1.37	-2.40	97.6	4.88	5	River water
0.90	-3.45	96.55	19.31	20	
G-SPR					
0.96	-2.60	97.40	4.87	5	Tap water
1.08	-2.85	97.15	19.43	20	
0.77	-2.80	97.20	4.86	5	River water
1.15	-2.15	97.85	19.57	20	

To evaluate the suitability of the proposed methods, the obtained results were compared with standard method [32]. The results obtained by the two different methods were statistically compared, using the Student t-test and F-test at 95% confidence level. In all cases, the calculated t- and F-values did not exceed the theoretical values (Table - 5), which indicate that there is no significant difference between either methods in terms of accuracy and precision. Table 6 shows the simple review for some reported methods were used for determination of BEN [33, 34].

Table-5: The comparison of the proposed rFIA methods with standard method.

Standard method		G-SPR		N-SPR		B-SPR		Methods
$(Xi - \bar{X})_2^2$	Rec % $(Xi)_1$	$(Xi - \bar{X})_1^2$	Rec % $(Xi)_1$	$(Xi - \bar{X})_1^2$	Rec % $(Xi)_1$	$(Xi - \bar{X})_1^2$	Rec % $(Xi)_1$	Sample
0.40	95.90	0.04	97.70	0.04	97.40	0.22	96.81	Pure
0.56	97.28	0.04	97.30	0.49	98.30	0.04	97.08	Tap Water
0.01	96.42	0.00	97.53	0.25	97.10	0.45	97.95	River Water
$((Xi - \bar{X})_2^2$ = 0.97	$(\bar{X})$ = 96.53	$((Xi - \bar{X})_1^2$ = 0.08	$(\bar{X})$ = 97.51	$((Xi - \bar{X})_1^2$ = 0.78	$(\bar{X})$ = 97.60	$((Xi - \bar{X})_1^2$ = 0.71	$(\bar{X})$ = 97.28	Statical values
S_2^2 = 0.49		S_1^2 = 0.04		S_1^2 = 0.39		S_1^2 = 0.35		
**t_{tab} = 2.776		0.26		0.44		0.42		*Sp
***F_{tab} = 19.0		2.33		1.97		1.41		t_{cal}
		12.25		1.27		1.40		F_{cal}

*S p = pooled standard deviation. Theoretical values at 95% confidence limit, n1= n2 = 3, **t = 2.776, where t has degrees of freedom = *(n1 + n2 – 2) = 4, ***F = 19.0, where F has degrees of freedom = (n1 – 1) = 2,(n2 – 1) = 2

Table-6: Review of some reported methods has been used for determination of BEN.

Ref	Linearity & LOD	Comment	Method
26	2.5–160 & 2.5 µg mL^{-1}	In the FI manifolds, the solutions of BEN (the latter after hydrolysis with NaOH) were injected into a diazonium ion carrier stream at pH 9.5 (buffered with tetrahydroborate), which was formed by mixing 2,4,6-trimethylaniline (TMA) with nitrate in a sodium dodecyl sulfate. Absorbance was measured at 390 nm.	FIA spectrophotometry
33	10-100 & 5.13 µg mL^{-1}	The method has been developed for the determination of BEN in water. The method is based on the reaction of hydrolyzed BEN with nitric acid to form a yellow complex with an adsorption maximum at 420 nm.	Spectrophotometry
34	1–150 & 0.738 µg mL^{-1}	The developed method is based on an alkaline hydrolysis of BEN in NaOH, and the resultant product was coupled with 2,4-dinitrophenylhdrazin in the presence of sodium periodate to form red-colored product which measured at 515 nm.	FIA spectrophotometry

Conclusions

The results presented in this paper clearly show that the rFI spectrophotometric method based on use of a lead dioxide solid-phase reactor (Bulk, Nanoparticles, and Grafted nanoparticles with 16 nm of SiO_2) for on-line oxidation coupling reaction. By comparing sensitivity, simplicity and sampling rate of proposed method are usually as good as those of methods previously reported but its flow manifold is much simpler. The reproducibility and stability of the solid-phase reactor make it an attractive alternative method for the determination of BEN in a flow system especially with use of PbO_2 nanoparticles and grafted nano PbO_2 by nano SiO_2 which gave more sensitivity in addition to more life time for N-SPR and G-SPR. Table -5 indicated that the results of analysis of these spiked samples are in good agreement with those obtained by the official method. Statistical analysis of the results obtained by FIA method and standard method using student t-test and the variance ratio F-test shows no significant difference between the proposed methods regarding accuracy and precision. Therefore the present methods may be suitable for routine determination of BEN in pesticide formulations and environmental samples.

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