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Pulse polarographic determination of benomyl fungicide in relation to its soil adsorption study

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Abstract

A differential pulse polarographic method for the determination of benomyl has been developed and which has been validated for soil adsorption study of this fungicide on four Indian soils of different characteristics. The method is based on the microwave assisted alkaline hydrolysis of the fungicide with sodium bicarbonate to carbendazim and n-butylamine, the letter upon reaction with carbon disulphide form n-butyldithiocarbamate which upon reaction with copper (II) perchlorate results into copper (III) dithiocarbamate complex which exhibit an analytical useful diffusion controlled peak at -94 mV (vs. SCE). Benomyl has been determined in the linearity range 1.45-14.56 µg mL^{-1}. The adsorption studies of this fungicide on four soils has been explained by using Freundlich's adsorption equation and the adsorption isotherms obtained are of L-type of Gile's classification. The high values of K_f, K_d and K_{oc} indicate that benomyl is strongly adsorbed on to soils which inhibits its penetration into water bodies. This is further substantiated by the Groundwater Ubiquity Score (GUS) which is in the range 0.30-0.37, classifying it as a non-leacher pesticide.

Keywords: Benomyl; microwave assisted hydrolysis, pulse polarography, copper (II) perchlorate and soil adsorption

1. Introduction

The pesticides are important tool for crop protection but the unregulated widespread use of these chemicals have resulted into environmental contamination and various negative effects on non-target organisms including human being [1-5]. Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) is one of the important benzimidazolic systemic fungicides used to control a wide range of fungal diseases of fruits, nuts, vegetables, field crops, turf, ornamentals and also for postharvest handling of crops [6-8]. Benomyl show many adverse effects viz. carcinogenic, reproductive, mutagenic and developmental abnormalities so it has been classified as hazardous to human health [8-12]. Applied pesticides will in one way or other end up in soil or water bodies. Soils and sediments are important sinks for pesticides because of their ability to sorb large amounts of these harmful compounds. Soil curtails the mobility of pesticide molecules in two ways: by sorption and by biological and chemical degradation. Benomyl and its metabolite Carbendazim adversely affects the soil enzyme activity [13]. Due to adverse effects of benomyl on non-target organisms and environment, its continuous monitoring and assessment is necessary so it is inevitable to find a simple, sensitive and reliable method for the determination of this fungicide in environmental samples. The methods used for benomyl determination are mostly based on its conversion to methyl 2-benzimidazole carbamate (MBC) commonly known as carbendazim or 2-aminobenzimidazole (2-AB) by acid/base hydrolysis. Of the various analytical methods used namely liquid chromatography [14-16], gas chromatography [17], flourometry [18], spectrophotometry [19, 20], spectroflourimetric [21] and immunosorbent assay [22] developed for the analysis of benomyl very less work has been done on its analysis by polarography [23] despite the fact that these methods are finding greater use in pesticide analysis because the determination can be done even at larger dilution and with smaller volume of solutions without any interference from inert carriers commonly present in formulated products. Mostly the above methods require number of steps with strict adherence to experimental conditions during the hydrolysis of this fungicide, thus making the method tedious and time consuming. On the other hand, liquid chromatographic methods are expensive.

In a significant effort in this context, we have been able to develop a remarkably sensitive differential pulse polarographic method for the determination of benomyl. The method has been applied for the analysis of benomyl in commercial formulation and has been validated for its adsorption study on four Indian solis. The formulation analysis is not only essential to ensure the quality of the marketed product but also to get reliable adsorption data. The various adsorption parameters viz. soil-adsorption coefficient (K_d), soil organic carbon partition coefficient (K_{oc}), Gibb's free energy (ΔG^o) and Groundwater Ubiquity Score (GUS) have been calculated.

2. Materials and Methods

2.1 Apparatus

All polarographic measurements have been made with an Elico (Hyderabad, India) polarographic analyzer (model CL-360) equipped with dropping mercury electrode (DME) as working electrode, saturated calomel electrode (SCE) as reference electrode and platinum wire as an auxiliary electrode. A microwave oven (Samsung, India) was used to carry out alkaline hydrolysis. A shaking incubator (Genei, Bangalore-India) was used in soil adsorption study.

2.2 Reagents and equipment

Acetonitrile (Merck) was kept over phosphorus pentoxide (5 g L^{-1}) and distilled twice. The analytical standard of Benomyl **(**Sigma-Aldrich Labochemikalien GmbH, Germany) was used and its stock solution (5×10^{-4} mol L^{-1}) was prepared in acetonitrile. A standard solution of copper (II) perchlorate (2×10^{-3} mol L^{-1} in acetonitrile) was prepared by the method described earlier[24]. Sodium perchlorate (Merck Schuchardt OHG, Germany) solution (10^{-1} mol L^{-1} in acetonitrile) was prepared by dissolving 12.24 g of pure compound in 1 L of acetonitrile. A 2% solution of carbon disulphide (Merck, AR) in acetonitrile was used. Sodium bicarbonate (CDH, LR), its 0.05 mol L^{-1} solution in distilled water was prepared. Triton-X100 (Merck Schuchardt OHG, Germany), 0.002% in acetonitrile was used as suppressor. Methyisobutylketone (MIBK) (SDFCL, AR) was used as an extracting solvent.

2.3 Soil samples

The soils used in the adsorption study were collected from Solan District of Himachal Pradesh, India. The soils samples were air-dried, crushed with wooden mortar and sifted through 2 mm sieve. Dried and sieved soil samples were placed in sealed glass jars and stored at room temperature.

2.4 Preparation of calibration graph for pure compound

Aliquots (0.1–2.5 mL) of standard solution of benomyl (5×10^{-4} mol L^{-1} in acetonitrile) were taken in 25 mL measuring flasks and volume made to 2.5 mL with acetonitrile. Each solution was mixed with 1.5 mL aqueous sodium bicarbonate (0.05 M) solution, 0.5 mL of 2% carbon disulphide (in acetonitrile), kept in a beaker containing 50 mL of water and then irradiated in the microwave oven for 40 seconds. The solution was allowed to stand for 15 minutes and then 2 mL of 2×10^{-3} mol L^{-1} copper(II) perchlorate (in acetonitrile) was added. The copperdithiocarbamate formed was extracted with 4 mL MIBK in the presence of sodium perchlorate (≈0.5g). The solution was dried over anhydrous sodium sulphate, transferred to polarographic cell, added 2 mL Triton-X100 (0.002% in acetonitrile) and final volume made to 20 mL with supporting electrolyte (0.1M sodium perchlorate in acetonitrile). Nitrogen gas was bubbled through the solution for 5 minutes and differential pulse polarogram of each solution was recorded at room temperature $(25\pm1)^0$ C with following instrumental parameters: initial potential = 100 mV; drop Time = 1 s; pulse amplitude = 50 mV, and scan rate = 6 mVs^{-1}. A calibration graph was constructed by plotting peak current (mA) (corresponding to -94 mV peak) vs concentration of fungicide benomyl (µg mL^{-1}) (Figure 1). The linear relationship between the concentration of the fungicide and the peak current is obeyed in the range 1.45 to 14.56 µg mL^{-1}.

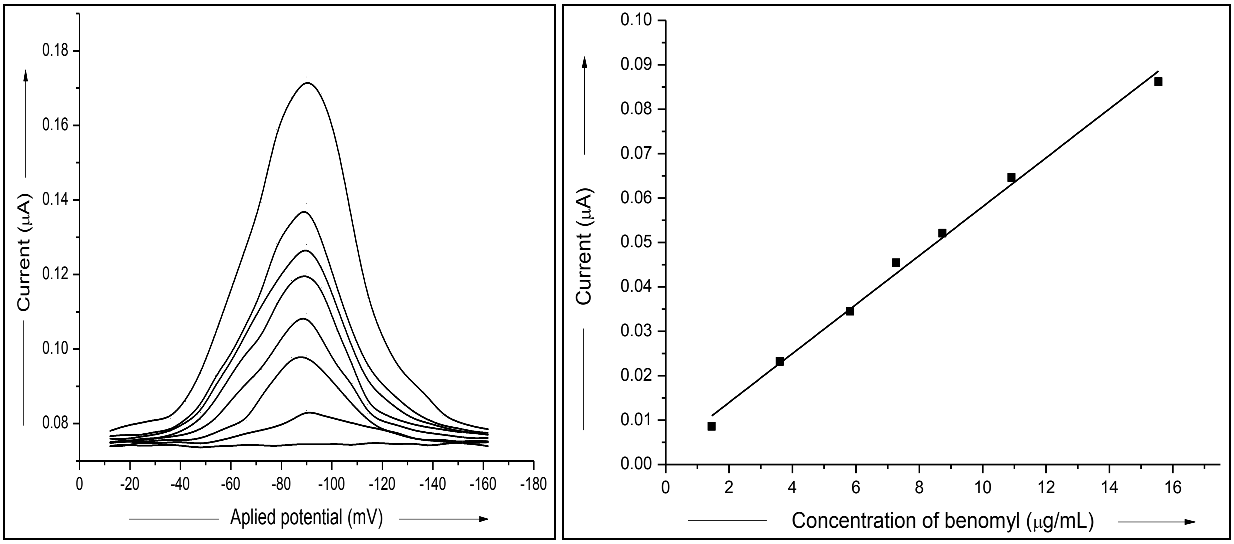


Fig 1: Relationship between current and concentration (calibration graph) for benomyl

2.5 Formulation analysis

A wettable powder formulation, Benlate-50, containing 50% active ingredient, procured from the authorised pesticide dealer was used. A single large sample of formulation equivalent to 10 mg active ingredient was shaken with 30 mL acetonitrile and filtered. The residue was washed three times with acetonitrile. The filtrate and washings were diluted to a known volume (50 mL) with the same solvent. Suitable aliquots (0.2-1.0 mL) of this solution were taken and processed for analysis as described above for the pure compound. Assay results are given in Table 1.

2.6 Soil adsorption study

Benomyl adsorption isotherms on four Indian soils of different soil characteristics (Table 1) were obtained by the batch equilibration technique using 50 mL conical flask. Triplicate soil samples (2.0 g) were equilibrated with aqueous-acetonitrile benomyl solutions in the concentration range from 43.5-101.6 µg mL^{-1} by shaking in Incubator Shaker PT-422 at room temperature (25±1°C) for 12 hour equilibrium time. After equilibration, the suspensions were centrifuged and the equilibrium concentrations (C_e) were determined in supernatants by the procedure described above for the pure compound.

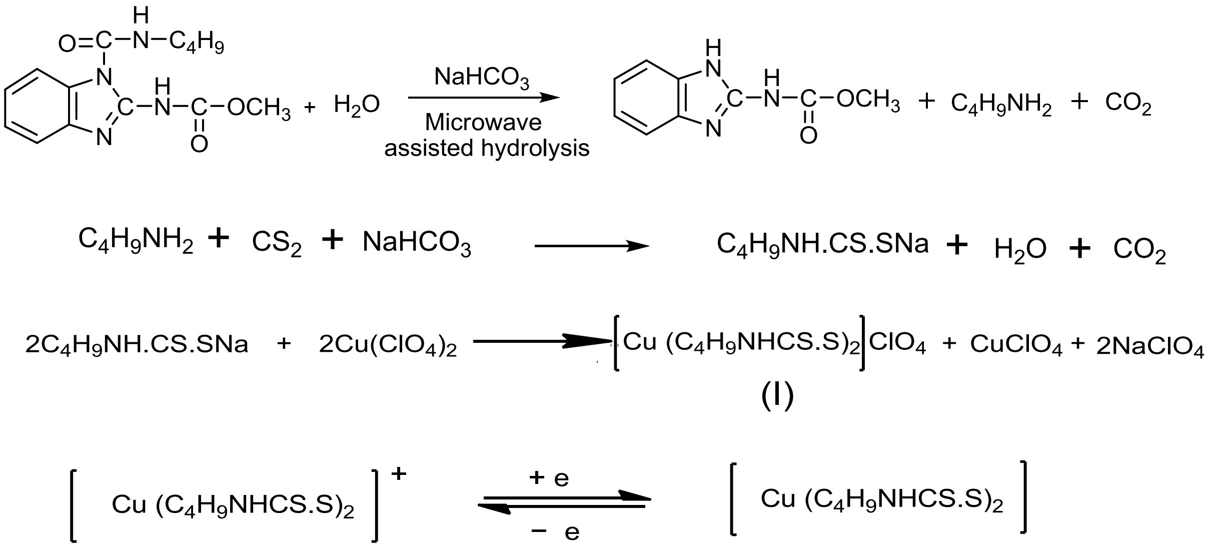
Table 1: Characteristics of the different Indian soils used in the adsorption study of benomyl.

Soil Sample	pH	Clay (%)	Organic carbon (%)	Cation Exchange Capacity (meq/100g)
I	7.2	32.6	0.8	13.1
II	7.6	18.2	0.9	12.9
III	6.5	20.0	1.5	11.0
IV	6.8	23.4	1.6	12.8

3. Results and Discussion

The differential pulse polarographic method for the determination of benomyl is based on the microwave assisted alkaline hydrolysis of the fungicide with sodium bicarbonate to carbendazim and n-butylamine which is quite well known [25]. Primary and secondary amines react with carbon disulphide in the presence of base to form corresponding dithiocarbamates has been thoroughly established in our laboratory [26]. That carbon disulphide transforms n-butylamine formed from the alkaline hydrolysis of benomyl into n-butyldithiocarbamate which reacts with copper (II) perchlorate to form copper (III) dithiocarbamate complex which exhibit a new diffusion controlled peak at -94 mV at DME, showing linear relationship between peak height and the concentration of amine as dithiocarbamate, has been made use of the pulse polarographic determination of benomyl. Excess carbon disulphide and methyl-2-benzimidazolecarbamate do not exhibit any peak at -94 mV; hence do not interfere in the method.

Copper (II) perchlorate in acetonitrile gives a well-defined diffusion controlled reduction peak at -300 mV (vs. SCE), whereas Benomyl gives a similar peak at 116 mV. When copper (II) perchlorate is added to hydrolysed benomyl solution in 1:1 molar ratio both the peaks due to copper (II) and benomyl disappeared completely and a new peak at -94 mV appeared showing much higher intensity. The results of analysis indicates that benomyl reacts with copper (II) perchlorate in 1:1 ratio to form most plausibly copper (III)-dithiocarbamate complex {I}, which has generated the new peak at -94 mV in differential polarography. The reaction taking place at the mercury electrode is believed to involve the reduction of copper (III)-dithiocarbamate complex undergoing one electron change. The most plausible mechanism for the proposed method is shown below:



The method has first been standardised on pure n-butyl amine before applying it to benomyl. The method when applied to the commercial formulations of benomyl gave recoveries in the ranges 97.9-99.2% of nominal content with RSD's in the ranges 0.52-0.82 % respectively (Table 2). The results have, however, been compared with an independent method [27].

Table 2: Assay of a commercial formulation of benomyl (Benlate) containing 50% active ingredient.

Active ingredient taken, µg	Active ingredient found* %	
	Present method	Comparison method[27]
2.0	98.1±0.72	97.8±0.80
4.0	98.6±0.82	98.3±0.94
6.0	97.9±0.68	96.7±1.01
8.0	99.2±0.52	98.5±0.86
10.0	98.8±0.74	98.2±0.80

*Values are mean ± standard deviation for three determinations.

The microwave assisted hydrolysis is advantageous then conventional heating in terms of higher reaction rates, greater selectibility and reproducibility of the results [28, 29]. The effect of time of hydrolysis and extracting solvent in the development of proposed method was studied before applying it to the analysis of benomyl in real samples. It has been observed best results were obtained corresponding to 40 sec hydrolysis time in a microwave. Of the various extracting solvents namely chloroform, methylisobutylketone (MIBK) and dichloromethane studied, the best results were obtained with MIBK. Since the solvent and supporting electrolyte can significantly affect the redox properties and voltammetric responses, the choice of proper solvent-electrolyte system becomes all the more important. Acetonitrile, an aprotic organic solvent, has been a solvent of choice from electrochemical point of view because of its convenient liquid range, ready availability, wide electrochemical range (from + 2.2 V to –3.1 V), moderate dielectric constant (facilitating the solubility of majority of organic compounds) and low toxicity. Of the various supporting electrolytes viz. lithium chloride, lithium perchlorate, sodium perchlorate, tetraethylammonium iodide and tetraethylammonium perchlorate studied; sodium perchlorate has been found suitable electrolyte in acetonitrile in the present study. Further copper(II) perchlorate shows good solution stability in acetonitrile and the resulting copper(I) perchlorate is stabilized by solvation and becomes resistant to aerial oxidation.

3.1 Soil adsorption study

The proposed method has been successfully applied to study the adsorption of benomyl in soils of different characteristics for the prediction of its movement in soil and aquifer. The adsorption of benomyl affects its bioactivity, mobility, persistence, toxicity as these processes are operative only on the unadsorbed fractions of pesticide [30]. As a result it influences the extent of surface and ground water contaminations. Adsorption of pesticide is generally evaluated using adsorption isotherms which represent a functional relationship between the amount of pesticide adsorbed and the pesticide concentration in solution at equilibrium [31, 32]. The adsorption isotherms of benomyl on four soils of different characteristics (Table 2) have been evaluated by Freundlich's adsorption equation (1) which is the most widely used in pesticide adsorption study.

$$X = K_f C_e^{n_f} \tag{1}$$

Where X is the amount of pesticide adsorbed mgkg^{-1} of the adsorbent; C_e is the equilibrium concentration in solution (mgL^{-1}). The adsorption isotherms have been obtained by plotting equilibrium surface excess concentration (X mg kg^{-1}) against the equilibrium solution concentration (C_e mg L^{-1}) and have been found to be of L-type (Figure 2) according to Gile's classification [33, 34]. The isotherms are used for identifying the nature of adsorption of solutes from aqueous solutions. L-type curve represents a high affinity between the solid surface and solutes at the initial stages of the isotherms and indicative of gradual decrease in sites availability for sorption as the concentration of solute in the solution increases. The adsorption coefficient K_f represents the amount of pesticide adsorbed at an equilibrium concentration of 1 mgL^{-1} and n_f represents the variation in adsorption with varying concentration of pesticide [30] and these are calculated from the least square methods applied to the linear form of the Freundlich's adsorption equation by the plot of log X versus log C_e (Figure 3) and results are summarised in Table 3.

$$\log X = \log K_f + n_f \log C_e \tag{2}$$

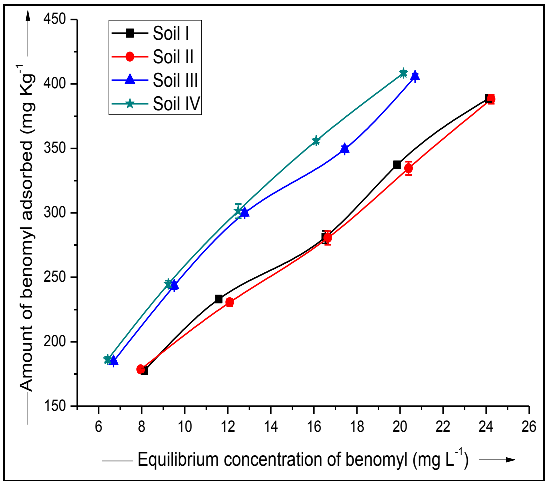


Fig 2: Adsorption isotherms of benomyl

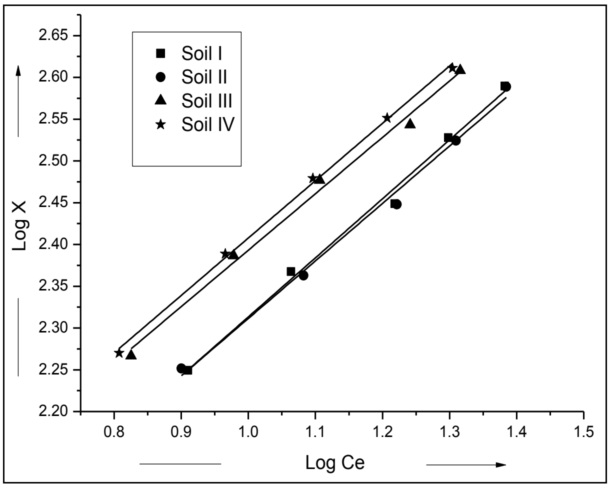


Fig 3: Plot of log X versus log C_e for the evaluation of Freundlich's adsorption coefficients K_f and n_f for benomyl.

The observed values of n_f indicate L-type isotherm and infer that there is minimum competition from solvent molecules for adsorption sites on the adsorbing surface. Higher the value of K_f, the lower is the tendency of pesticide to move in soil, as has been found in the present case. The various adsorption parameters for benomyl viz. soil-adsorption coefficient (K_d), soil organic carbon partition coefficient (K_{oc}), Gibb's free energy (ΔG^o) and Groundwater Ubiquity Score (GUS) have been calculated by using equations 3-6 respectively [35, 36] and are presented in Table 3.

$$K_d = \frac{X}{C_e} \tag{3}$$

$$\Delta G^\circ = -RT \ln K_d \tag{4}$$

$$K_{oc} = K_d \times \left(\frac{100}{\% O.C.} \right) \tag{5}$$

$$GUS = \log t_{1/2} \left[4 - \log(K_{oc}) \right] \qquad (6)$$

Where R = gas constant, T = absolute temperature, $t_{1/2}$ = pesticide persistence (half life), OC = organic carbon content of soil.

Table 3: Adsorption parameters for the adsorption of benomyl fungicide on four Indian soils.

Soil	K_f	n_f	K_d	K_{oc}	ΔG^o	GUS
I	40.43	0.70	18.41	2321	-7.218	0.30
II	43.98	0.68	18.15	2017	-7.183	0.33
III	52.22	0.67	23.26	1551	-7.798	0.37
IV	52.45	0.72	24.39	1524	-7.916	0.36

The organic matter content and the clay content of soils play a very important role in the sorption of pesticides on to the soil. A great proportion of pesticide molecules is adsorbed by soils high in soil organic carbon content and or high clay content because organic matter or clay provide soils with an increased number of adsorptive sites onto which pesticides molecules can bind. Pesticides with a high water solubility are generally adsorbed to a lesser degree than pesticides with low water solubility. This is explained on the basis of cation exchange capacity (CEC) of the soil which gives hydrophobic nature of adsorbent, so greater the value of CEC of soil, its surface will be more hydrophobic and will adsorb hydrophobic organic pesticides to greater extent [37]. The value of K_d represents the extent of adsorption and in general higher the K_d value, the greater is the pesticide adsorption [30]. The K_d for a pesticide is soil-specific and vary with soil texture and its organic matter content but the soil organic carbon partition coefficient (K_{oc}) is less soil specific and is calculated by normalizing adsorption coefficient (K_d) with the organic carbon (OC) content of the soil. The high values of K_d and K_{oc} suggest that benomyl is strongly adsorbed to soils and it is immobile in the soils and don't leach to ground water. This is further confirmed by evaluating the leaching potential in terms of GUS index which has been determined by using experimentally observed K_{oc} value for each soil sample and literature reported half life of benomyl [8]. The GUS values for benomyl ranged from 0.30-0.37, which are far below 1.8, classifying it as a non-leacher pesticide. GUS score is used for predicting the leaching behaviour of compounds. Generally they can be classified as leacher (GUS>2.8), transition (2.8>GUS<1.8) and non-leacher (GUS<1.8) [38]. Therefore, the use of benomyl does not represent the real hazard to ground water contamination. The negative value of Gibb's free energy (ΔG^o) for the adsorption of benomyl suggests that the adsorption process is energetically favourable.

4. Conclusion

To reduce hazards posed by pesticides to human health and environment, their determination and monitoring is essential. The proposed pulse polarographic method for the determination of benomyl is simple, rapid and reliable. Adsorption studies of benomyl indicate that it is strongly adsorbed on to soils and this consequently inhibits its penetration into water sources. This is further supported by Groundwater Ubiquity Score (GUS) which is far below 1.8, classifying it as a non-leacher pesticide and hence it does not represent hazard to ground water contamination.

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