Use of a Stopped-Flow Pneumatic Mixing Module To Analyze Dinitrophenol Pesticides. Simultaneous Determination of Dinoseb and Dinobuton

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A kinetic study of the degradation of dinobuton in an alkaline medium has been undertaken by using a stopped-flow pneumatic system. A semiautomatic method for determining dinobuton is proposed for the first time. A detection limit of 0.40 μg mL^{-1} was calculated. Simultaneous determination of dinobuton and dinoseb can be made by combination of equilibrium and kinetic measurements. The proposed method has been applied to analyze binary mixtures of dinobuton and dinoseb samples with the amount of both components simulating the composition of one undegraded and several degraded dinobuton samples. Also, the procedure has been tested in the analysis of a commercial formulation of dinobuton and the results validated with high-performance liquid chromatography (HPLC).

Keywords: *Dinitrophenols; dinobuton; dinoseb; stopped-flow; commercial formulations*

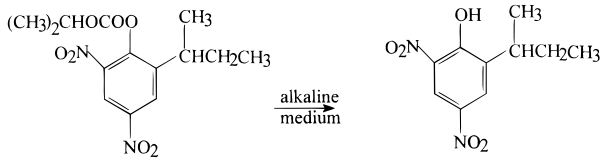
INTRODUCTION

Dinobuton (2-*sec*-butyl-4,6-dinitrophenyl isopropyl carbonate) is a nonsystemic acaricide and fungicide active against powdery mildews; it is recommended for glasshouse and field use against the mites of the red spider and powdery mildews of apples, cotton, and vegetables at 50 g a.i./100 L Dinoseb (2-*sec*-butyl-4,6-dinitrophenol) is a contact herbicide used as the ammonium or an amine salt or acetate ester. Solutions of dinoseb in oil are used for control of annual weeds in beans, peas, and potatoes. Both compounds are dinitrophenol derivatives. Dinobuton and dinoseb are formulated with tetradifon (4-chlorophenyl-2,4,5-trichlorophenyl sulfone) and monolinuron (3-(4-chlorophenyl)-1-methoxy-1-methylurea), respectively (Worthing, 1987).

Several methods have been published for determining both dinobuton and dinoseb. The most classical method uses photometry with a previous separation step. Hence, Lynch's method (Lynch, 1976) involves preliminary desterification of dinobuton to dinoseb on neutral alumina; the dinoseb is then eluted from the column as its butylammonium salt and determined spectrophotometrically. Through the efforts of the Dinitro Pesticides Panel, adaptations were made for formulations and the method was subjected to international collaborative trial. Methods for analysis of technical pesticides formulation of DNOC (4,6-dinitro-*o*-cresol) and dinoseb were later published (Sánchez-Rasero, 1980; Heniriet, 1981). An independent check of the results was made by high-performance liquid chromatography (HPLC) analysis of DNOC, dinoseb, and dinobuton. No statistical difference was found between the spectrophotometric and the HPLC results (Farrington et al., 1982).

An international collaborative study has been published (Farrington et al., 1983) to check the results for the determination of the active ingredient in formulation, in technical DNOC and dinoseb, and in technical dinobuton. Separation steps for free dinoseb are necessary before dinobuton can be determined. More recently, HPLC and GC methods for dinocap (2-(1-methylheptyl)-4,6-dinitrophenyl crotonate), dinoseb, dinoterb (2-*tert*-butyl-4,6-dinitrophenol), and DNOC have been accepted by General Inspectorate for Health Protection and published as Analytical Methods for Pesticide Residues in Foodstuffs (Ministry of Public Health, 1996). The most simple proposed method involves an alkaline hydrolysis step to give the corresponding phenols, and later the determination by reversed-phase ion-pair HPLC with UV detection at 365 nm.

In this paper, a stopped-flow mixing system coupled with a photometric detector has been applied for simultaneous determination of dinoseb and dinobuton. The hydrolysis reaction of dinobuton to give dinoseb according to the following reaction was applied:



Dinobuton (246 nm) **Dinoseb** (375 and 420 nm)

Absorbance measurements at zero reaction time were combined with kinetic measurements of the initial rate of the hydrolysis reaction of dinobuton. Physicochemical parameters were studied to optimize the kinetic procedure. The purpose of this work was to develop a stopped-flow system for the semiautomatic determination of dinobuton and dinoseb in routine analysis of formulations to avoid the previous separation steps of dinoseb described in the bibliographic procedures. The combination of equilibrium and stopped-flow measurements allows us simultaneous direct determination.

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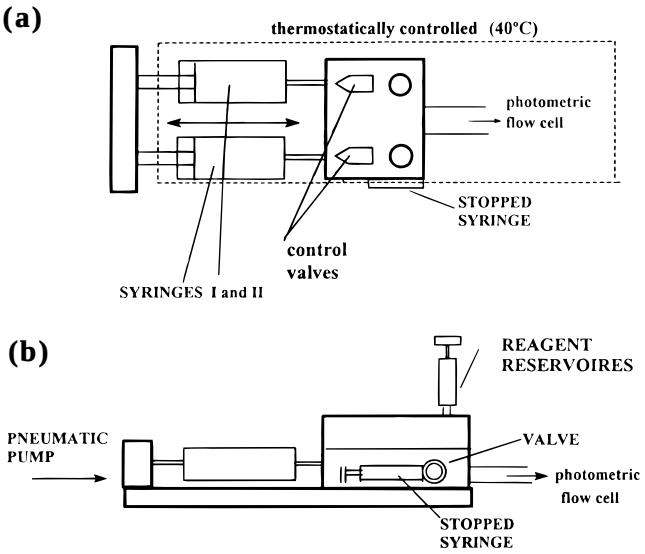
Scheme 1. (a) Top and (b) Side Views of the Pneumatic Mixing Module, RX-1000 Rapid Kinetics Accessory[a]



[a] Syringe I, aqueous−ethanolic (50%) pesticide solution; syringe II, aqueous−ethanolic (50%) 1 M potassium hydroxide solution.

EXPERIMENTAL PROCEDURES

Apparatus. An RX-1000 Rapid Kinetics accessory provided with a Pneumatic Drive (3.5 bar pressure regulator) (Applied Photophysics Limited, UK) was used. The accessory contains two 2.5-mL, pneumatically driven reagent syringes, a silica flow cell (10-mm path length), an efficient twin-jet mixer, and a liquid-crystal display (LCD) of temperature with 0.11 °C precision. At the end of a stopped-flow drive, the stopping syringe piston causes the trigger microswitch contacts to close, and this is used as a means to trigger a signal digitizer (Scheme 1). A Beckman DU 640i spectrophotometer, provided with the software package, was used for all measurements, acquisition of kinetic data, storage, and analysis of spectrophotometric data. A thermostatically controlled bath, Selecta Unitronic 320 OR, was used for temperature control of both the spectrophotometer and the rapid kinetic accessory.

Reagents. Stock dinoseb and dinobuton solutions were prepared by dissolving 0.010 g of certified dinoseb and dinobuton solid product (Dr. Ehrenstorfer Reference Materials, distributed by Scharlau, Barcelona, Spain) in 100 mL of absolute ethanol. All chemicals and solvents used were of analytical reagent grade. The commercial formulation Acarelte-40 EC (Probelte, Murcia, Spain) was obtained from a local commercial company. The use of gloves and hood is essential when pesticides are handled.

Procedures. *General Procedure for Determining Dinoseb and Dinobuton*. An aqueous ethanolic (50%) solution containing a volume of dinobuton and/or dinoseb standard solution at a final concentration up to 30 μg mL^{-1} was used to fill one of the two 2.5-mL drive syringes (syringe I) of the stopped-flow module. The second drive syringe (syringe II) was filled with a solution containing a 50% aqueous ethanolic 1-M potassium hydroxide solution. The solution contents in syringe II were used as a blank of the photometric measurements. For the sample analysis in each run, 0.15 mL of each solution was mixed and injected in the flow cell by using a 3.5 bar pressure. The evolution of the absorbance at 420 nm was recorded during 60 s, maintaining a constant temperature (40 °C). Dinoseb content was determined by measuring the absorbance at zero reaction time and using the appropriate calibration graphs. Dinobuton was determined by measuring the rate of the reaction as the tangent to the kinetic curves during the first 10 s of the hydrolysis reaction and using the appropriate calibration graphs.

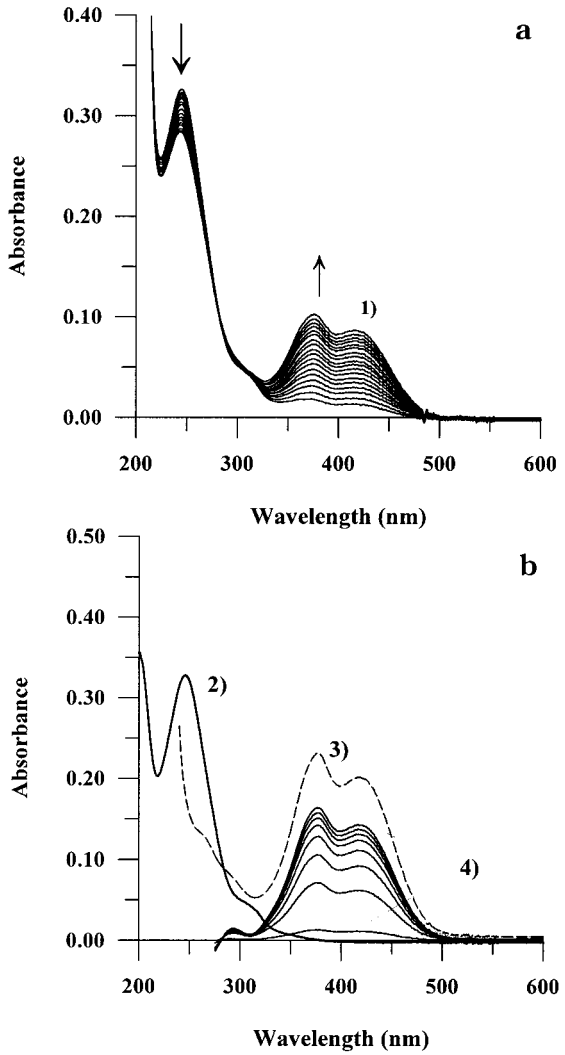


Figure 1. (a) (1) Evolution of the absorption spectrum of a pH = 10 aqueous−ethanolic (1:1) dinobuton solution. One spectrum each 15 s. (b) (2) Absorption spectrum of an aqueous−ethanolic dinobuton solution. (3) Evolution of the absorption spectrum of a 0.25 M KOH aqueous−ethanolic (1:1) dinobuton solution; one spectrum each 15 s. (4) Absorption spectrum of a 0.25 M KOH aqueous−ethanolic (1:1) dinoseb solution.

Determination of Dinobuton in a Commercial Formulation. Acarelte 40-EC (Probelte) is a liquid commercial formulation with a theoretical composition: 40% dinobuton and excipient. An Acarelte stock solution was prepared by adding 250 μL of the formulation (previously homogenized) in a 250-mL volumetric flask and diluting to the mark with ethanol. Aliquots (between 0.25 and 2.50 mL) of this stock solution were placed in a 25-mL flask and a sufficient volume of ethanol to give a final ethanol−water mixture 50+50 (v/v) was added and diluted to the mark with water. The samples were then analyzed by the general stopped-flow proposed method.

RESULTS AND DISCUSSION

As stated in the literature, dinobuton in alkaline medium exhibits a hydrolysis reaction influenced by several physical−chemical parameters, such as temperature and basic medium. The product generated in this reaction shows a bathochromic displacement of the absorption maximum with respect to that of dinobuton. In Figure 1a, the evolution of the absorption spectrum of an aqueous ethanolic dinobuton solution (obtained in a static photometric cell) in basic media is shown. The generated product shows absorption maxima at 375 and

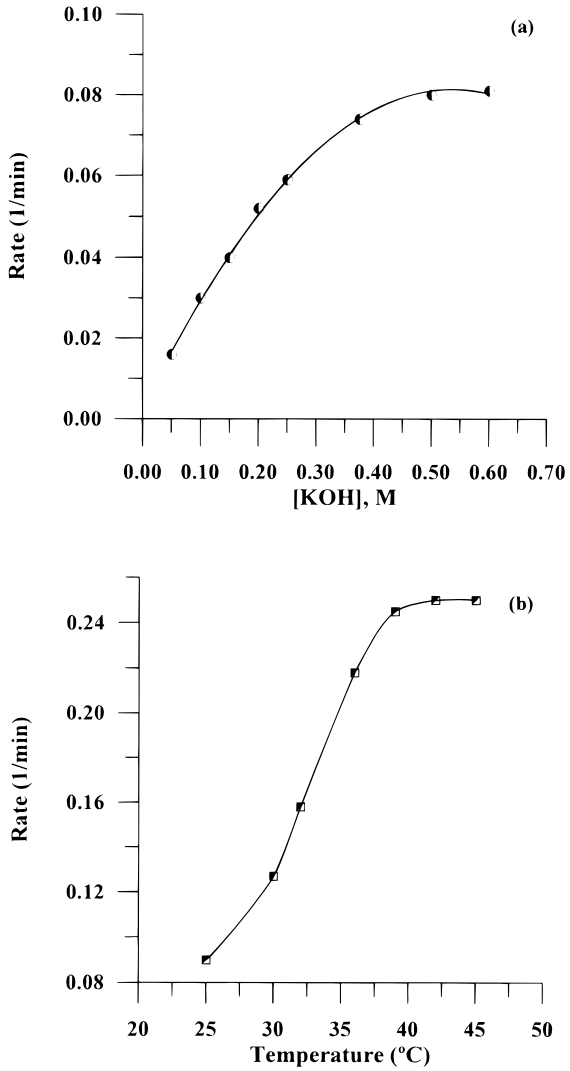


Figure 2. (a) Influence of potassium hydroxide concentration on the rate of the hydrolysis reaction (temperature 25 °C; period of measurements = 0−10 s). (b) Influence of the temperature in the rate of the reaction ([KOH] = 0.5 M).

420 nm, and the absorption maxima located at 246 nm in the initial dinobuton disappears. It must be noticed that the final absorption spectrum obtained in this evolution is similar to the absorption spectrum of a basic dinoseb solution. Also, the absorption spectrum of an aqueous ethanolic dinoseb solution in basic medium is shown (Figure 1b).

Kinetic Behavior. The evolution of the absorbance at 420 and 375 nm with the time corresponds to the formation of dinoseb. The kinetic curve exhibits a linear plot up about 10 s, and for reaction times higher than 60 s, the signal remains constant. Kinetic data were obtained from the initial rate of formation of dinoseb by measuring the tangent to the kinetic curves obtained at $\lambda = 420$ nm. The influence of several variables in the rate of the reaction was studied. The partial order for each variable was calculated from the resulting log−log plots.

Influence of Physical−Chemical Variables. A kinetic study of the influence of several variables on the rate of reaction was made in order to develop a kinetic method for the determination of dinobuton alone or in mixture together with dinoseb simulating a contaminated dinobuton. In the first, syringe I of the stopped-flow system was filled with an aqueous ethanolic (50%)

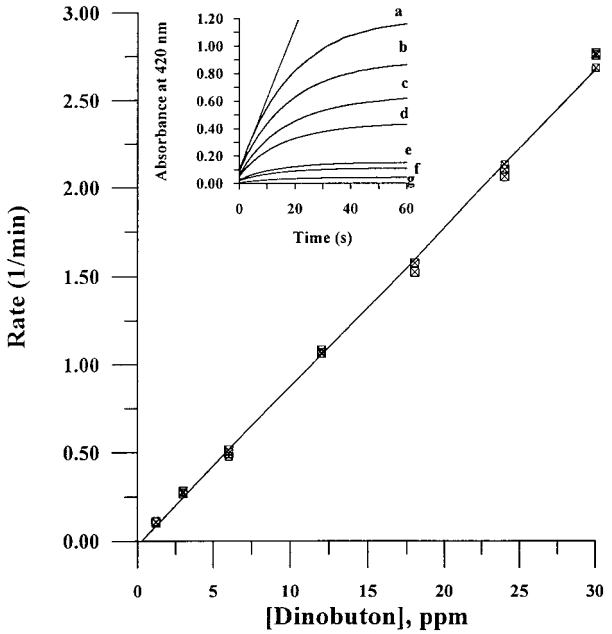


Figure 3. Kinetic curves obtained for different amounts of dinobuton: (a) 30 μg mL^{-1}; (b) 24 μg mL^{-1}; (c) 18 μg mL^{-1}; (d) 12 μg mL^{-1}; (e) 6 μg mL^{-1}; (f) 3 μg mL^{-1}; (g) 1.2 μg mL^{-1}.

solution containing 7 μg mL^{-1} of dinobuton (final concentration in the flow cell, 3.5 μg mL^{-1}), and syringe II was filled with an aqueous ethanolic (50%) solution containing different concentrations of KOH. The influence of the KOH concentration was studied in the range 0.05−0.60 M as the final concentration in the flow cell. In Figure 2a, the influence of the concentrations of KOH in the rate of reaction is represented. For [KOH] < 0.4 M, the rate of reaction increases (+1 partial order) when we increase the concentration of KOH, and for [KOH] > 0.45 M, the rate of reaction remains constant (0 partial order). A 0.5-M final KOH concentration was selected as sufficient to obtain the highest sensitivity.

The effect of temperature was examined between 25 and 45 °C. The two drive syringes and flow cell were simultaneously thermostatized. The rate of the reaction is strongly favored with the increase in the temperature, and for temperature values higher than 38 °C it remains constant. The variations in the rate of reaction at different temperatures are represented in Figure 2b. A plot of the logarithm of the rate constant against the inverse of the absolute temperature allows us to calculate an activation energy of 12.13 kcal mol^{-1} K^{-1}. A temperature of 40 °C was selected as adequate for the later experiments. At the selected temperature the absorbance due to dinoseb formation remains constant with the time.

Calibration Curves and Analytical Parameters. Absorbance-time stopped-flow signals at 420 nm were recorded between 0 and 60 s for solutions containing different amounts of dinobuton or dinoseb. No evolution with the time was observed for any of the dinoseb standard samples assayed. Calibration graphs were constructed by measuring the absorbance at 420 nm versus concentration of dinoseb. Calibration curves for dinobuton were obtained by using the evolution of the absorbance at 420 nm versus concentration of dinobuton. The period of measurement was selected by application of a least-squares method to the kinetic curves. The most favorable statistical parameters for kinetic measurements were obtained in the range between 0

Table 1. Analytical and Statistical Parameters for the Determination of Dinobuton and Dinoseb at 420 nm

determination of dinobuton	determination of dinoseb
equation	equation
rate (min^{-1}) = $-2.21 \times 10^{-2} + 9.34 \times 10^{-2}$ dinobuton	$A^{t=0} = 7.78 \times 10^{-3} + 3.92 \times 10^{-2}$ dinoseb
range of application: 0.5−30 μg mL^{-1}	range of application: 0.3−30 μg mL^{-1}
relative standard deviation of the slope: 4.44×10^{-4}	relative standard deviation of the slope: 6.74×10^{-4}
relative standard deviation of the intercept: 1.36×10^{-2}	relative standard deviation of the intercept: 4.13×10^{-3}
correlation coefficient (r): 0.9997	correlation coefficient (r): 0.9982
measurement period: 10 s	measurement period: at zero reaction time
temperature: 40 °C	temperature: 40 °C

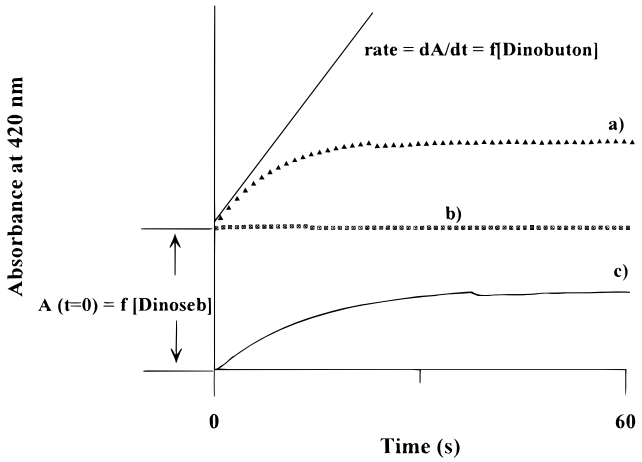


Figure 4. Kinetic curves obtained for (a) binary mixture of dinobuton and dinoseb (5.44 + 5.04 μg mL^{-1}); (b) dinoseb (5.04 μg mL^{-1}); and (c) dinobuton (5.44 μg mL^{-1}).

and 10 s. The calibration graph (rate versus [dinobuton]) is linear up to 30 μg mL^{-1} for Dinobuton (Figure 3). The limit of detection, according to the Clayton criterion (Clayton et al., 1987) is 0.40 μg mL^{-1} ($\alpha = \beta = 0.05$; $n = 3$) . Linearity was obtained between the absorbance at 420 nm, at zero reaction time, and the concentration of Dinoseb up to 30 μg mL^{-1}. The limits of detection by Clayton criterium was 0.20 μg mL^{-1}. Statistical parameters and analytical characteristics of the determination of dinobuton and dinoseb are summarized in Table 1.

Simultaneous Determination of Dinobuton and Dinoseb. The possibility of simultaneously determining dinoseb and dinobuton was tested. In Figure 4 the kinetic curves obtained at 420 nm for dinobuton, dinoseb, and the respective binary mixture are shown. The influence of the concentration of dinobuton and dinoseb in the absorbance at zero reaction time and the concentration of dinobuton in the rate of the reaction were analyzed. The following equations can be applied:

$$\text{rate (1/min)} = 0.0934[\text{dinobuton}] - 0.0221$$

$$A^{t=0} = 0.0040[\text{dinobuton}] + 0.0392[\text{dinoseb}] + 0.0078$$

The above-mentioned equations have been applied to simultaneous determination of dinobuton and dinoseb in two prediction sets, A and B. Set A was constructed simulating undegraded and degraded dinobuton for different decomposition grades. Set B was composed of pure and contaminated dinobuton with different amounts of dinoseb, using two different concentrations of dinobuton. In Table 2 the composition of set A, the respective decomposition grades, and the relative errors are summarized. Least-squares methods have been applied to analyze the relation actual versus predicted for both dinobuton and dinoseb. Correlations of 1.059 and 1.001

Table 2. Simultaneous Determination of Dinoseb and Dinobuton in a Degraded Dinobuton (Set A)

dinobuton		rel	dinoseb		rel	decomp
actual (μg mL^{-1})	predicted (μg mL^{-1})	error (%)	actual (μg mL^{-1})	predicted (μg mL^{-1})	error (%)	grade (%)
14.52	14.63	+0.7	–	–	–	0
13.07	13.22	+0.1	1.02	1.04	+2.45	10
11.62	11.56	−0.5	2.14	2.24	+4.91	20
10.17	10.24	+0.8	3.26	3.37	+3.4	30
7.26	7.58	+4.4	5.35	5.63	+5.3	50
4.36	4.42	+1.61	7.49	8.03	+7.2	70
2.91	2.81	+3.3	8.61	8.98	+4.3	80

Table 3. Simultaneous Determination of Dinobuton and Dinoseb in a Contaminated Dinobuton

dinobuton		rel	dinoseb		rel	contam
actual (μg mL^{-1})	predicted (μg mL^{-1})	error (%)	actual (μg mL^{-1})	predicted (μg mL^{-1})	error (%)	grade (w/w) × 100
5.60	5.54	+1.1	0.73	0.74	+2.0	13
5.60	5.39	−3.7	1.46	1.40	−4.4	26
5.60	5.69	+1.7	1.82	1.76	−3.0	33
5.60	5.96	+6.5	2.55	2.57	+1.0	46
5.60	6.06	+8.3	3.64	3.51	−3.4	65
5.60	6.18	+10.4	5.46	5.17	+5.2	97
10.16	9.33	−8.1	1.02	1.01	−0.9	10
10.16	9.91	−2.5	3.06	3.32	+8.7	30
10.16	9.81	−3.5	6.12	6.45	+5.4	60

Table 4. Determination of Dinobuton in a Commercial Formulation (Acarelte 40-EC)

	dinobuton (%)	RSD (%) ($n = 9$)
proposed method	40.8	2.3
HPLC	37.5	8.3
manufacturer's specification	40	–

were obtained for dinoseb and dinobuton, respectively. In Table 3 the composition and the obtained results for set B are shown. Dinobuton samples contaminated up to 65% can be analyzed. In Figure 5, actual versus predicted concentration plots for dinobuton and dinoseb in set B are shown. Linear plots are observed up to a total concentration (dinobuton plus dinoseb) of 5×10^{-5} M, and for higher total concentrations positive errors are obtained. The real dinobuton samples must be adequately diluted to minimize errors.

Determination of Dinobuton in a Commercial Formulation. Dinobuton was determined in the commercial agricultural formulation Acarelte. The results are summarized in Table 4 and are compared with those obtained by HPLC (Farrington et al., 1982). Concordant results were obtained. A statistical comparison between the mean values and precision parameters obtained by application of the HPLC reference procedure and by application of the proposed method (Válcarcel and Rios, 1992) was made. No statistical differences were observed by using $a = 0.05$ ($n = 9$). On the other hand, no decomposition of the commercial product was detected.

Influence of Foreign Species. Several pesticides present in commercial formulations (tetradifon) and

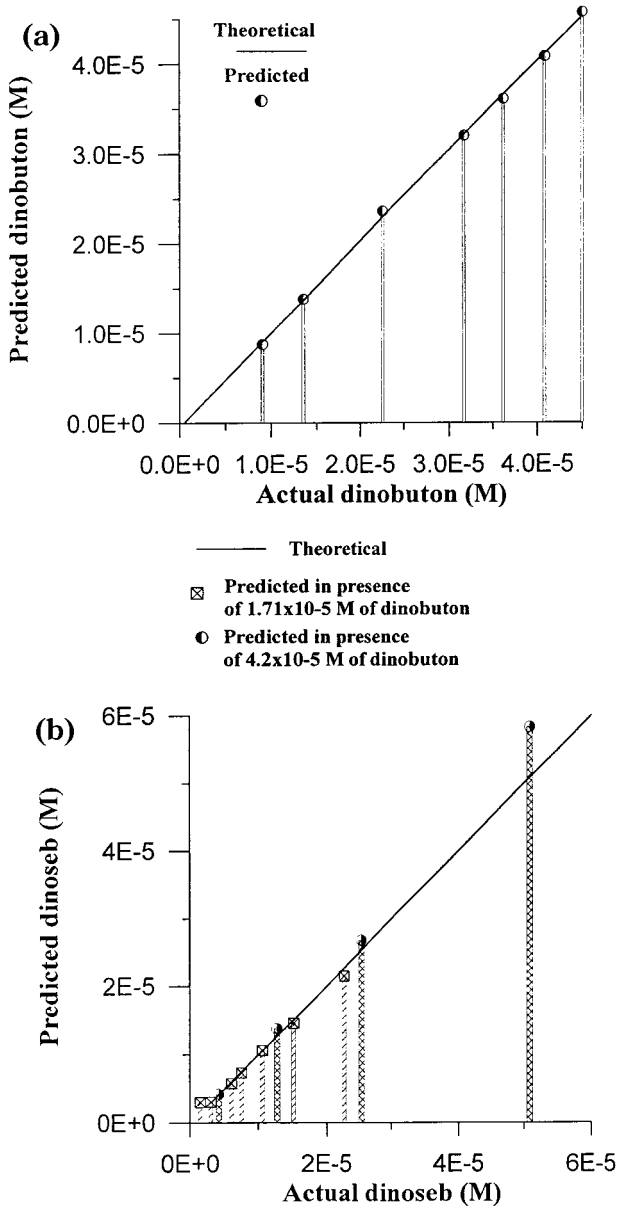


Figure 5. Actual vs predicted plot for (a) dinobuton and (b) dinoseb in presence of (○) 2.33×10^{-5} M and (▲) 4.66×10^{-5} M of dinobuton.

others with similar chemical structures (dinoterb, dinoseb, and DNOC) have been tested as interference species in the kinetic determinaton of dinobuton. Tetradifon, dinoterb, and DNOC were tolerated at least for a 10:1 (interference:analyte) w:w ratio, for a total concentration of interference + analyte not greater than 10^{-4} M. Dinoseb was tolerated at least for a 20:1 (interference:analyte) w:w ratio, but the total concentration of dinoseb + dinobuton must be smaller than 5×10^{-5} M. In rotuine analysis of commercial formulations, the samples must be adequately diluted.

Conclusions. The proposed method combines the kinetic measurements to determine dinobuton with measurements of the absorbance at zero time to determine dinoseb. The total analysis time per sample (or standard) is drastically reduced to only 10 s, while an analysis time of 10 min per sample is necessary in most of the recommended chromatographic methods. Permit us to determine decomposition grade of in a commercial dinobuton. Also, the analysis of technical dinobuton contaminated with dinoseb can be made without prior separation steps of the dinoseb. The sensitivity of the proposed method is adequate for application in routine analysis of manufactured product but is not sufficient to determine this pesticide in enviromental samples. In the last case, preconcentration steps similar to those used in the HPLC methods could be applied.

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