Kinetic Method for the Determination of 2,4-Dinitrophenol

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A kinetic method for the determination of dinitrophenol is proposed. The method is based on the inhibiting effect of 2,4-dinitrophenol on the Mn(II) catalysis of the oxidation of malachite green with potassium periodate. The reaction was monitored spectrophotometrically at 615 nm. Kinetic expressions for the reaction are postulated. The optimal experimental conditions for the determination of 2,4-dinitrophenol were established and 2,4-dinitrophenol was determined in concentrations from 0.092-0.92 μg . mL^{-1} with relative standard error of 5.9 %. Detection limit is 0.014 μg . mL^{-1}. The selectivity of the method is appropriate. The method was applied for the determination of dinitrophenol in urine and river water.

Key words: 2,4-dinitrophenol, kinetic determination, malachite green oxidation, potassium periodate

Toxic polynitroaromatic compounds, such as 2,4,6-trinitrophenol and 2,4-dinitrophenol, contaminate the environment. These compounds have been extensively used in chemical industry for the synthesis of dyes, explosives in the past and pesticides. Pesticides are applied widely to protect plants from diseases, weeds and insects damage, and this is why they usually come into contact with soil and water, where they undergo variety of transformations, that provide a complex pattern of metabolites. Pesticides in soil continue to be studied more than other environmental contaminants, because they are widely used to control pest that affect agricultural crops and pest in the home, yards and gardens. There is also an increasing interest in their transformational products, because they can be present at higher levels in soil than the parent pesticide itself.

In Europe, the EU Directive 91/414 I EEC and its subsequent amendments establish that, before placing a pesticide on the market, environmental data must be provided for all amounts of metabolites, and degradation and reaction product, which account for more than 10% of the amounts of the active substance [1].

The fate of pesticides in soil is controlled by chemical and physical dynamics of this matrix. These processes can be grouped into those that affect persistence, including chemical and microbiological degradation, and those that affect mobility involving sorption, plant uptake, volatization, wind erosion, run-off and leaching.

Chemical and microbiological processes degrade pesticides. Chemical degradation occurs through reaction such as photolysis, hydrolysis, oxidation and reduction [2, 3]. Biological degradation takes place when the soil microorganisms consume or break down pesticides [4, 5]. These organisms are mainly distributed in the top centimeters of the surface layer of the soil, where the organic matter acts as a food supply [6].

The development and the application of methodologies for the determination of pesticides and their metabolites is a challenging task. Analysis of pesticides is generally carried out by gas chromatography or liquid chromatography coupled to different detectors [7, 8] especially to mass spectrophotometers [9, 10]. However, alternative and/or complementary methods using capillary electrophoresis [11], biosensors and bioassays have emerged recently [12].

In this paper a sensitive and simple kinetic method for the determination of 2, 4-dinitrophenole (DNP) is proposed. The oxidation of malachite green (MG) with potassium periodate in an acetate buffer solution gives a colorless product. The reaction is catalyzed by the traces of Mn(II) and it is used for its kinetic–catalytic determination [13]. On the other side, the rate of the reaction decreases proportionally with increasing the concentration of 2,4-dinitrophenol. That means that small amounts of 2,4-dinitrophenole strongly inhibit the catalysis of this reaction. This fact was used as the base of the kinetic method for determining ultra micro amounts of 2, 4-dinitrophenol.

Materials and method

Apparatus

A Perkin-Elmer Lambda 15 Spectrophotometer, connected to a thermo-circulating bath was used for the absorbance measurements. The absorbance of solution was measured at 615 nm every 30s over a period of 7 min from the beginning of the reaction. Hanna instruments pH – meter was used to measure the *p*H values of the solution. Sigma buffers *p*H 7.00±0.01 and *p*H 4.00±0.01 were used for the calibration of the pH-meter.

The solutions were thermo-stated at 22 ±0.1°C before beginning of the reaction.

Reagents

A stock MG solution (1 . 10^{-3} M) was prepared by dissolving MG in deionized water. The working MG solution (4 . 10^{-5} M) was obtained by diluting the stock MG solution in water, and was used within 3 weeks after the preparation. The periodate solution (1. 10^{-3} M) was prepared by dissolving potassium periodate in deionized water.

The acetic acid solution (10 M) was prepared from 99% reagent. The Mn(II) solution (1 . 10^{-5} M) was prepared by dissolving manganese chloride in deionized water. The 2, 4-dinitrophenol (1. 10^{-3} M) solution was prepared by dissolving 2,4-dinitrophenol in deionized water.

All chemicals reagents were of analytical grade and were provided by Merck unless indicated otherwise. Solutions were prepared by deionized water.

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All the stock solutions were stored in polyethylene containers. Working solutions of periodate, Mn(II) and 2,4-dinitrophenol were prepared immediately before use.

All the polyethylene containers and glassware used were cleaned with aqueous HCl (1:1) and then thoroughly rinsed with deionized water.

General procedures

Procedure for calibration curve

Aliquots of MG solution and acetic acid, and catalyst Mn(II) and inhibitor (2,4-dinitrophenol) solutions were transferred into the 10 mL volumetric flask and accurate volume of the working solution of periodate was added and diluted up to 10 ml with deionized water. The contents of the mixture were well shaken and immediately transferred to the spectrophotometric cell. The absorbance was recorded as a function of time at 615 nm against a reagent blank at 22±0.1C°. The rate of the reaction is proportional to the slope of the curve that shows logarithm of the absorbance as a function of concentration. Calibration graph was obtained by plotting the initial rate of the reactions vs. the molar concentration of 2,4-dinitrophenol.

Following concentrations of 2,4-dinitrophenol were used for obtaining the calibration graph: 0.092, 0.184, 0,386, 0.552, 0.736, 0.920 M. Concentrations of other components of the system were:

$c_{MG}=1.6 \cdot 10^{-6}M$, $c_{CH3COOH}=2.5$, $c_{Mn(II)}=2 \cdot 10^{-6}M$,

$c_{KIO4}=2 \cdot 10^{-4}M$

Procedure for the determination of 2,4-dinitrophenol in urine and river water by kinetic method

2,4-dinitrophenol was added to 20 mL of freshly collected urine (river water) sample.

2,4-dinitrophenol was distilled from nonvolatile impurities using atmospheric distillation with Graham borsilicate condenser. The volume of the distilled 2,4 – DNP was 20 mL, the same as the initial volume. It was expected that the distillate volume would be ultimately equal that of the original sample [14]. 10 mL of distilled 2, 4-dinitrophenol solution was diluted to 100 mL with deionized water.

(1mL = 0.5 μg 2,4-dinitrophenol in urine, 1 mL = 0.6 μg 2,4-dinitrophenol in river water). After these treatments 1 ml of each of these samples was used for the procedure recommended in this paper.

Procedure for the determination of 2,4-dinitrophenol in urine and river water by standard method

In 1mL of distilled solution of 2,4-dinitrophenol was added a little Zn powder and 2 mL of sulphuric acid (1:5). Solution was transferred into the test tube with glass stopper through which goes a small bore glass tube. After 15 min solution was filtrated and 0.5 mL of 0.5% potassium dichromate was added and solution filled up to 10 mL with deionized water. Absorbance of the solution was then measured at 429 nm. Concentration of DNP was determined using standard calibration line [15]. According to the different laboratory conditions (apparatus and level of purity of used substances) it was necessary to establish and adjust calibation graph recommended in above mentioned literature. The obtained calibration graph (fig. 5) had the following linear equation: $A = 0.185 \cdot c_{2,4\ DNP}$. Standard deviation of the method was $S = 1.98 \cdot 10^{-3}$ and relative error was G = 4.17% for determination of microgram concentrations of 2,4 – dinitrophenol.

Results and discussions

Kinetic studies

The kinetic of catalytic reaction of potassium periodate and malachite green, both in the presence and absence of 2, 4-dinitrophenol was investigated by following the changes of tgα (α = dA/dt) when varying the concentration of one component while keeping the concentration of all the others constant. Figures from 1 to 4 show the effect of each component on both the catalytic and inhibited reaction.

Figure 1 shows the influence of concentrations of acetic acid on the rate of both reactions. It can be seen that the greatest difference between the reaction rates occurs at the acetic acid concentration of 2.5 M, while 2,4-dinitrophenol maximally decreases the catalytic reaction rate. It appears From figure 1 that there is a complex relationship between the acetic acid concentration and the catalytic reaction rate, i.e. the order of the reaction is

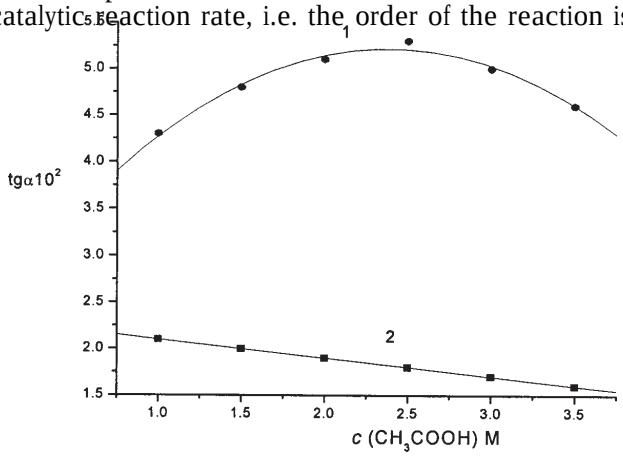

Fig. 1. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on acetic acid concentration. Initial concentrations: c_{MG} = 1.6 . 10^{-6} M, $c_{Mn(II)}$ = 2 . 10^{-6} M, c_{KIO4} = 2 . 10^{-4} M, c_{DNP} =0.92μg/mL^{-1}, T = 295K

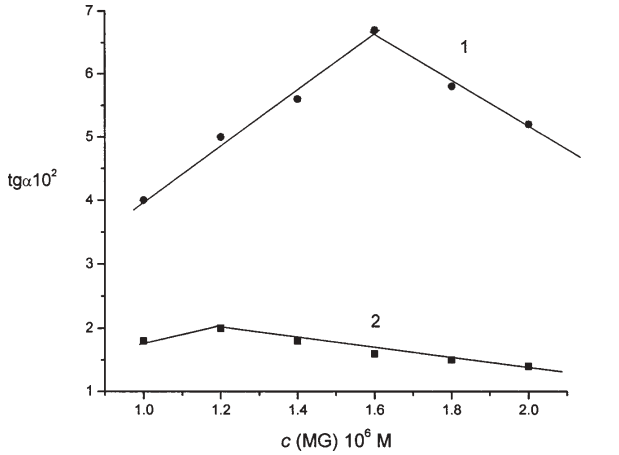

Fig. 2. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on MG concentrations. Initial concentrations: $c_{CH3COOH}$ = 2.5 M, $c_{Mn(II)}$ = 2 . 10^{-6} M, c_{KIO4} = 2 . 10^{-4} M, c_{DNP} =0.92μg/mL^{-1}, T = 295K

variable with respect to the hydrogen-ion concentration for the range of concentration studied. For further work the concentration of 2.5 M for acetic acid was selected.

The dependence of the reaction rate on the concentration of MG is shown in figure 2. in which it can be seen that the inhibited reaction is first order with respect to the MG concentration up to 1.2 . 10^{-6} M and negative first order with respect to the higher concentrations, where the catalytic reaction is first order with respect to the MG concentrations up to 1.6 . 10^{-6} M, and negative first order for the higher concentrations. The maximal difference

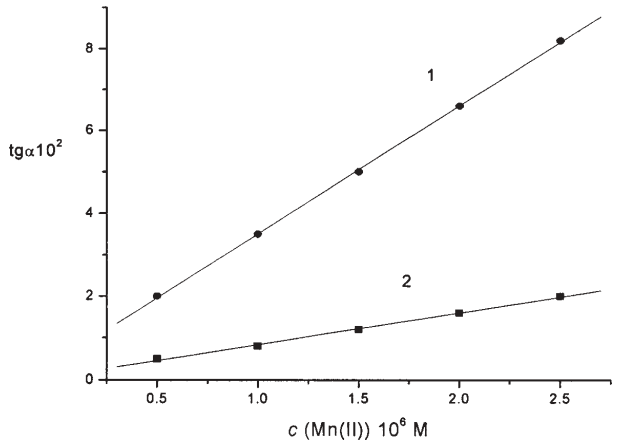

Fig. 3. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on Mn(II) concentrations. Initial concentrations: c_{MG} = 1.6 . 10^{-6} M, $c_{CH3COOH}$ = 2 .5 M, c_{KIO4} = 2 . 10^{-4} M, c_{DNP} =0.92μg/mL^{-1}, T = 295K

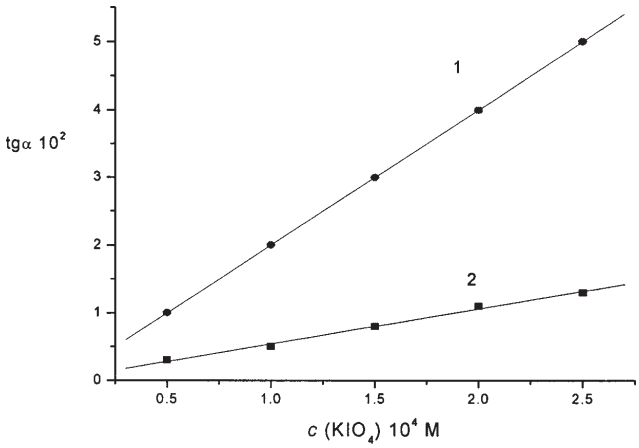

Fig. 4. Dependence of the rate of the catalyzed (1) and inhibited (2) reaction on the KIO_4 concentrations. Initial concentrations: c_{MG}=1.6 .10^{-6}M, $c_{CH3COOH}$ = 2.5 M, $c_{Mn(II)}$ = 2 . 10^{-6} M, c_{DNP} =0.92μg/mL^{-1}, T = 295K

between the reaction rates in the presence and absence of dinitrophenol occurs at MG concentration of 1.6 . 10^{-6} M. This concentration was optimal and used in further work.

It may also be seen from figure 3 and figure 4. that the reaction rates increase with increasing concentration of the catalyst and potassium periodate. Both reactions are first order with respect to the catalyst and potassium periodate concentrations. For further work the catalyst and periodate concentrations of 2.0 . 10^{-6} M and 2.0 . 10^{-4} M were used, respectively, because at higher concentrations the linear part of the kinetic curve (log A-t) is rather short.

Under the optimal reaction conditions: the dinitrophenol concentration was varied from 0.092-0.92 μg . mL^{-1}.

A linear dependence was established between tgα and the concentration of dinitrophenol:

$$tg\alpha = -0{,}0416 \cdot c_{DNP} + 0.061 \quad (r = 0.998) \qquad (1)$$

r-coefficient of correlation

for dinitrophenol concentration range is 0.092-0.92 μg . mL^{-1}.

On the basis of all the obtained results, the following kinetic expressions were derived for the proposed indicator reaction in the absence and in the presence of dinitrophenol, (at the constant *p*H)

$$-d(MG)/dt = k_1 \cdot c_{MG}^{-1} \cdot c_{Mn(II)} \cdot c_{KIO_4} \qquad (2)$$

k_1 is constant proportional to the rate constant of the catalyzed reaction (for the first part of the curve: 1.0-2.5 M) and

$$-d(MG)/dt = k_2 \cdot c_{MG}^{-1} \cdot c_{Mn(II)} \cdot c_{KIO_4} \cdot c_{DNP}^{-1} \qquad (3)$$

k_2 is constant proportional to the rate constant of the inhibited reaction.

The above equations are valid for the following concentration ranges:

c_{MG} from 1.6 . 10^{-6} M to 2.0 . 10^{-6} M (eq.2), and from 1.2 . 10^{-6} M to 2.0 . 10^{-6} M (eq.3), c_{KIO4} from 0.5 . 10^{-4} M to 2.5 . 10^{-4} M, (eq.2 and 3), $c_{Mn(II)}$ from 0.5 . 10^{-6} M to 2.5 . 10^{-6} M (eq.2 and 3).

Table 1

LINEAR CONCENTRATION RANGE. ACCURACY AND PRECISION OF THE METHODS FOR DETERMINATION OF 2, 4 – DINITROPHENOL

Method	Concentration range DNP (μg·ml^{-1})	Relative standard deviation	t_{exp}
Kinetic	0.092-0.92	3.2	0.92(t=2.77)
Spectrophotometric	0.5-5.0	7.0	1.21(t=2.77)

Table 2

TOLERANCE LEVELS OF INTERFERENCE IN THE KINETIC DETERMINATION OF 0.184 μg .mL^{-1} OF DINITROPHENOL USING THE OPTIMAL CONDITIONS

Tolerance level c_{ion}/c_{DNP}	ion added
10^4	Na^+, Cl^-, K^+
10^3	NO_3^-, CH_3COO^-, PO_4^{-3},F^-
10^2	Mg^{2+}, Sr^{2+}, SO_4^{-2},Br^-,Ca^{2+}
10	HCO_3^-,$H_2PO_4^-$, Cu^{2+},Cd^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, Al^{3+},Hg^{2+}, Se^{4+},
1	Pb^{2+}, Fe^{3+},$C_2O_4^{2-}$,SCN^-,citrate, interfere

Analytical characteristics

Under the optimized conditions given above, the calibration graph was prepared using standard DNP solution, for standard spectrophotometric and kinetic-spectrophotometric methods. The calibration graphs are linear in the concentration ranges indicated in the table 1 [16]. The accuracy and precision of the two methods applied for 0.74μg dinitrophenol/ml are included in the table 1, from which it is concluded that both the kinetic and the spectrophotometric method have a negative systematic error and that they are both accurate and precise. The kinetic method is more simple and precise than the spectrophotometric method.

The limit of detection can be calculated using the equation 4:

$$LOD = 3S_b/m = 0.014\ \mu g\ .mL^{-1} \quad (4)$$

The limit of quantification can be calculated using the equation 5:

$$LOQ = 10S_b/m = 0.047\ \mu g\ .mL^{-1} \quad (5)$$

sample	2,4-DNP ($\mu g \cdot ml^{-1}$)			
	taken	Found [a]		
		Kinetic method	Recovery (%) for kinetic method	Spectrophoto-metric method	Recovery (%) for spectrophotometric method
Urine	0.5	0.486[a]±0.052[b]	95.4	0.484±0.082	88.2
River water	0.6	0.538[a]±0.058[b]	93.7	0.572±0.073	89.8

[a]Mean of 5 determination ($\bar{x}$)

[b] S_b

Table 3
RESULTS FOR THE DETERMINATION OF 2, 4 - DINITROPHENOL USING KINETIC AND SPECTROPHOTOMETRIC METHODS

Sample matrix	Preparation method	Analytical method	Sample limit detection	Percent recovery	reference
serum	Extract sample in sodium chloride-sodiumcarbonate with metilethyl ketone	spectrophoto metric	2 $mg \cdot L^{-1}$	No data	Gehring and Buerge, [20]
Urine	Reflux sample in sulphyric acid with ethyleter, evaporate organic solvent and dissolve residue in dilute sodium hydroxide	polarographic	10 $mg \cdot L^{-1}$	No data	Gisclard Woodward, [21]
Ground water	Extract acidified sample with methylen chloride, concentrate	GC-FID Method 8270	13 $\mu g \cdot L^{-1}$	78	EPA [22]
Water and waste water	Extract acidified sample with methylen chloride, concentrate	GC-MS Method 625	42 $\mu g \cdot L^{-1}$	78 to water and 108 for waste water	EPA [23]
Water	Extract acidified sample with methylen chloride, concentrate	HPLC-UV	0.025 $\mu g \cdot L^{-1}$	88	Schultz [24]
Soil	Extract with methanol in rotary shaker, centrifuge	HPLC-UV	0.1 $mg \cdot kg^{-1}$	93-94	Bouchard [25]
Urine	DNP was added into freshly collected urine sample. DNP is distilled from non-volatile impurities. Solution was diluted with deionized water.	Kinetic-spectrophoto metric method	0.092 $mg \cdot L^{-1}$	95.4	Mitic et al., presented paper
River water	DNP was added into the river water sample. DNP is distilled from non-volatile impurities. Solution was diluted with deionized water	Kinetic-spectrophoto metric method	0.092 $mg \cdot L^{-1}$	93.7	Mitic et al., presented paper

Table 4
ANALYTICAL METHODS FOR DETERMINATION OF DINITROPHENOL IN REAL SAMPLES

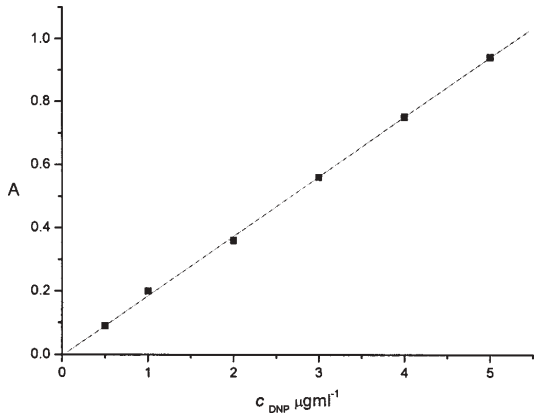

Fig. 5. Adjusted calibration graph for the spectrophotometric determination of 2, 4 - dinitrophenol

S_b - standard deviation for the reaction in the absence of 2,4-dinitrophenol; m-the slope of the calibration graph [17, 18].

Interference study

The interference of several foreign ions upon 2S (S-standard deviation) criterion [19] is determined at the constant concentration of 2,4 - dinitrophenole(0.184 μg. mL^{-1}). In table 2 tolerance levels of anions and cations usually found in real samples are presented. Many of cations and anions do not interfere at ratio of foreign ion to dinitrophenole of 1:10 and 1:100, respectively. The influence of some aromatic hydroxyl compounds was also investigated. The presence of each of resorcinol, pyrogallol, phenol and catechol interfere in the ratio 1:1.

Application of this method for 2, 4-dinitrophenol in urine and river water

Results, obtained applying the proposed kinetic method, as well as the spectrophotometric method, for the determination of 2,4-dinitrophenol in urine and river water samples are shown in table 3. The results were reproducible as indicated by standard deviation values. The accuracy of the method was valuated by statistical comparison of the results with those obtained by the spectrophotometric method.

Conclusion

A few different analytical methods for determination of DNP are given in the table 4. HPLC and GC chromatography with different detectors used for the determination of 2,4-dinitrophenol show high sensitivity and selectivity, but suffer more or less time-consuming procedure and/or expensive and complicate instrumentation. Few spectrophotometric and kinetic-spectrophotometric method for determination of trace level of the dinitrophenol have also been published using various types of indicator reactions. Most of these methods have a narrow dynamic range of determination and are applicable only to miligram amounts. In this respect, the present procedure shows that the Malachite Green-Mn(II)-potasiumperiodate system can be successfully used for the determination of trace amounts of 2,4-dinitrophenol in river water and urine at normal temperature with a minimum analysis time and without using activators and surfactants. The novel method is quite sensitive, precise, rapid and selective and therefore can be used to determine microgram amounts of 2,4-dinitrophenol.

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