SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 3, pp. 581-587, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

SPECTROPHOTOMETRIC ASSESSMENTS OF METHYL PARATHION IN WATER SAMPLES

*MS Gaur, Amit Kumar Sharma, Pradeep Sharma, Vipin Mishra and Rajeev Kumar Tiwari
Department of Physics, Hindustan College of Science and Technology
Farah, Mathura-281122 (UP), India.

ABSTRACT

A simple, sensitive and selective spectrophotometric method for the assessment of methyl parathion in water samples through various formulations is described. The method is based on the coupling of methyl parathion (MP) in alkaline conditions which gives the greenish yellow to lime yellow colored product having the maximum absorption at 395nm.The product is stable for 4 hours. The Beer's limit in the concentration range of 0.1µg ml^{-1} to 1.5 µg ml^{-1} has been observed. The molar absorptivity was found to be 0.047L mol^{-1} cm^{-1}. The recovery of MP in distil water have been found to be less than tap water.

Keywords: Methyl parathion, hydrolysis, UV-VIS spectrophotometer, pH, temperature.

INTRODUCTION

Organophosphorous (OPs) insecticides are widely used in agriculture for the control of various insects. These compounds generally have higher acute toxicity than chlorinated insecticides, which is due to the inhibition of the enzyme cholinesterase, and essential component of animal nervous system (Lai *et al.,* 1995; Gallo and Lawryk, 1990). The persistence of organophosphorous insecticides in aquatic environments is affected by several factors, such as oxidation, photolysis and biological degradation. Chemical hydrolysis of organophosphorous insecticides was reported to play an excellent role in the persistence of these compounds in the vapor, and soil moisture all provides sample opportunity for hydrolysis (Eichelberger and Lichtenberg, 1971; Gomma *et al.,* 1969). Methyl parathion can under go oxidative degradation to give less paraoxon, in the presence of ultraviolet radiation. Hydrolysis of methyl parathion occurs in different conditions but it is more rapid under alkali conditions (Kuo and Perera, 2000). The hydrolysis and biodegradation was studied in four types of water (ultra pure, pH 6.1; river water, pH 7.3; filtered river water, pH 7.3; and sea water, pH 8.1) maintained at 6ºC and 22ºC in the dark by Zheng and Liu (2002) and Serdar and Gibson (1985). They reported the half-lives of methyl parathion at 6ºC in the four types of water were determined to be 237, 95, 173 and 233 days, and at 22ºC, the half lives were 46, 23, 18 and 30 days respectively. Methyl parathion parathion provides an excellent structural template in order to observe the influence of oxidation on hydrolysis rate. We have noticed the chemical hydrolysis of methyl parathion and further identified the various groups in hydrolyzed product at 30ºC. In order to determine the reaction mechanism more accurately we have identified and measured the rate of appearance of hydrolysis products.

*Corresponding author email: mulayamgaur@rediffmail.com

The organophosphorus pesticide is capable of detoxification a variety of neurotoxins by hydrolyzing various phosphorus-ester bonds (P=O, P-F, P-CN, and P=S) between the phosphorus center and an electrophilic leaving group, in which the leaving group is attached to the phosphorus center via a sulfur atom (Huang *et al.,* 2004; Jan *et al.,* 2003; Dannenberg and Pehkonen, 1998; Munnecke, 1976; Farooq *et al.,* 2004) as shown figure 1.

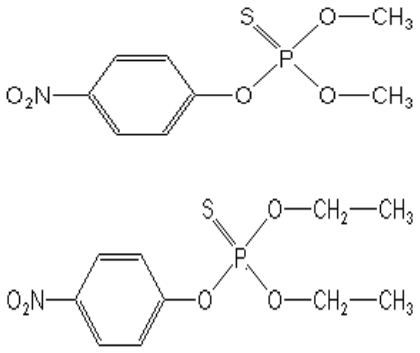


Fig. 1. Structure of Methyl parathion and Parathion.

The present investigation describes a simple procedure by coupling of known amounts of methyl parathion in alkaline conditions and its spectroscopic study for standardization. The qualitative and quantitative estimation of methyl parathion in their formulations of fortified water (both tap & distilled water) were determined using their spectral data.

Experimental Details

Methyl parathion (98% pure), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium sulphate anhydrous, methanol (HPLC grade), n-hexane (HPLC grade) and buffer capsules of different pH were

purchased from Merck, Germany. Cflorosil (60-100 meshes) was obtained from CDH laboratory, India. All other chemicals are used analytical reagent grade.

A Hitachi U-2800 double beam spectrophotometer (Tokyo, Japan) was used for recording UV-VIS spectra and absorption measurements.

MATERIALS AND METHODS

Sample Preparation

Due to low solubility of methyl parathion in water, a stock solution of methyl parathion of 10 ppm was prepared in 99% absolute methanol. MP samples of 0.1 ppm to 1.5 ppm were prepared using serial dilution method with the help of micro-pipettes (eppendorf-AG, Hamburg Germany). Aqueous solution of buffer solutions (pH-4, 9), sodium hydroxide crystal (0.1M) and hydroxyl ammonium chloride crystal (0.05M) were prepared with milli Q-water as a hydrolyzing agent for chemical hydrolysis of MP.

Tap water was collected (500ml) from Hindustan College of Science and Technology, Farah, Agra-Mathura Highway, India. All samples were spiked by adding known amounts of methyl parathion concentration which lies within the Beer's law range (1.5-0.1ppm) in this proposed method. A stock solution of methyl parathion of 10 ppm was prepared in 99% absolute methyl alcohol by using serial dilution method. After one minute 100µl of strong base 0.1M NaOH was added and pH of samples was adjusted by 0.05 M hydroxylamonoium hydrochloride (50µl) after few seconds 1.5 to 0.1 ppm was produce lime yellow color as a result of hydrolysis. Hydrolysis was not only carried out at room temperature but also elevated up to 40ºC temperature. The final hydrolyzed product was tested by color group test and analyzed by UV-VIS spectrophotometer. We were found that the absorption of hydrolysis sample increases with increase of concentrations of methyl parathion. The absorption was taken at 395nm wavelength.

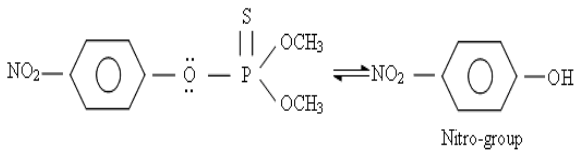
RESULTS AND DISCUSSIONS

Spectral characteristics of Methyl Parathion in Formulation

The proposed method involves the coupling reaction of methyl parathion with hydrolyzed product under alkaline conditions of hydrolysis process and gives lime yellow colour to dark coloured product having maximum absorption at 395 nm. This wavelength was used for all measurements. The absorption spectrum of the reaction product (10ppm) formed is shown in figure 3.

Effect of pH on Hydrolysis

Chemical hydrolysis of methyl parathion and hydrolysis products p-nitrophenol and dimethyl thiophosphoric acid was investigated by using colour group test. Hydrolysis proceeded at higher rates under alkali condition, suggesting that the reactions were more effectively catalyzed by strong nucleophile hydroxide ions (OH^-), this was more apparent for nitro aromatic compound in p-nitrophenol. In this observation we were found that pH is slightly affected with concentration of methyl parathion along with few hour of time. The hydrolysis of MP was observed in different value of pH (i.e. pH; 7, 8 9 and 10) for hydrolyzing agent but results of pH 9 are found to be showing appreciable variation of colour intensity with respect to concentration.



Hydrolysis in Alkali medium

Effect of Sampling Time and Temperature in Colour Stability of Methyl Parathion.

Under the optimization condition, the lime yellow colored product was found to be stable for 12 h. Reproducible results were obtained at the temperature range of 20-40ºC, figure 6 (A= 20ºC, B= 30ºC and C= 40ºC). An increasing in temperature above 40ºC, the curve between absorbance of hydrolyzed samples with concentration does not linear, but the minimum sampling time for all measurement was taken 10 min each. However, a temperature of 30ºC and hydrolysis of methyl parathion and its hydrolysis products are fundamentally different at pH 9. At alkali thiophosphoric acid, the formation of the p-nitro phenol is presumably due to the combined attack of OH^- ion at the P atom and S (thio group associated with double bond), which results in cleavage of the P=S bond and obtained paraoxon P=O (due to oxidation) in intermediated cases. Sampling time of 10 minutes is recommended for this proposed process.

With the help of nucleophile substitution reaction the mechanism of hydrolysis can be explained (Fig. 2). Medium of (i.e. pH 9), the methyl parathion formed phenol derivatives p-nitrophenol (nitro aromatic compound) and dimethyl thiophosphpric acid.

Mechanism of Hydrolysis

In this observation, we found that the reaction mechanism was concerned with nucleophilic substitution reaction. Methyl parathion and dimethyl thiophosphoric acid are phosphate esters. The hydrolysis of methyl parathion occurs through reaction with a strong nucleophile (OH^-) by necleophile substitution reaction (SN_2). The reaction mechanism rate of SN_2 reaction depends both on the concentration of the nucleophile and concentration of the electrophile, it is a bimolecular reaction. The mechanism depends on another essential property of the electrophile,

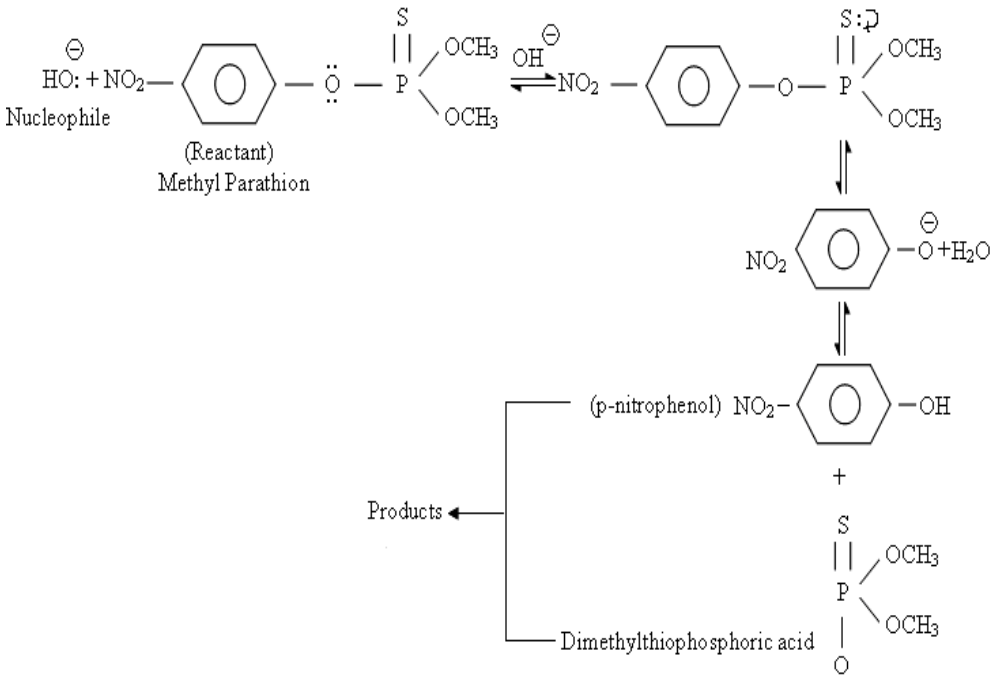


Fig. 2. Hydrolysis pathways reaction of methyl parathion during colorimetric process.

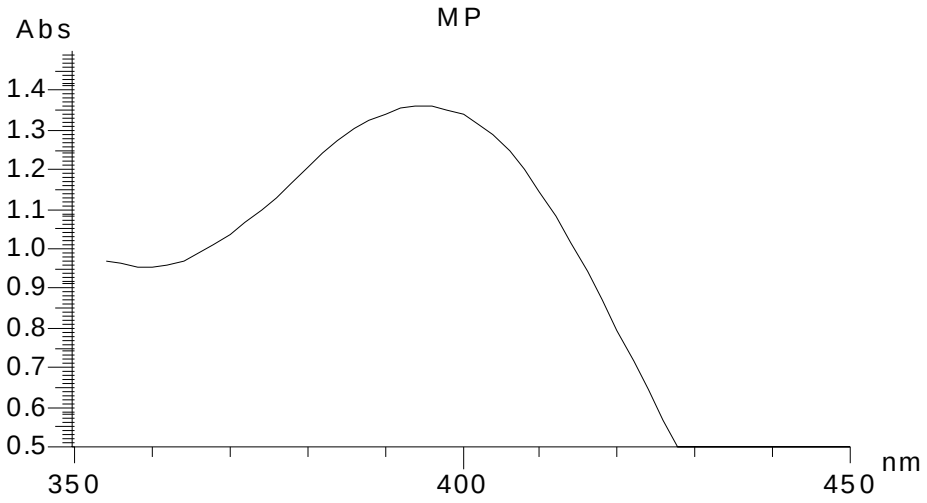


Fig. 3. UV-VIS Spectra of MP.

ability of the central atom and the presence of leaving group. The concentration of strong nucleophile greatly effects the hydrolysis mechanism if we double the concentration of nucleophile, the reaction rate doubled and we double both, the reaction rate becomes quadruples. The proposed reaction mechanism is shown as under:

Functional Group Test

During hydrolysis process the essential steps of group identification in water formulations have been taken. The group identification test of methyl parathion has to be performed with the help of various reagents as shown in test table 1.

Quantification of Methyl Parathion

According to Beer's law, colored product of hydrolyzed methyl parathion was determined by all measurements of absorbance at 395 nm for a set of solutions containing varying amounts of analyte and specified amounts of reagents against blank solution. Limit of quantification (LOQ) is determined by taking the ratio of standard deviation of the blank with respect to water and the slope of the calibration curve multiplied with time limit of

Table. 1. Test table.

Functional group	Diagnostic test	Reagent	Result	Conclusion	Comments
1.Phenol	Solubility test	5% dil.NaOH and saturated $KHCO_3$	soluble in dil.NaOH, insoluble in saturated $KHCO_3$	Weak acid: Phenol	May be phenolic group.
	Ferric Chloride test	ferric chloride	colored complex (red)	Phenol	No
	Pauly test	diazonium salt	colored azo compounds	Phenol	No
2.Nitro	-	ferrous hydroxide	color change from blue to red brown	Nitro	No
	-	titanous chloride	pink solution looses color	Nitro	No
3.Ester	Hydroxamic test	hydroxylamine	deep purple colored complex salt	-	May be amides also.
	Acid test	hydrochloride then ferric chloride	yellow to brown color precipitate	Ester	Positive result

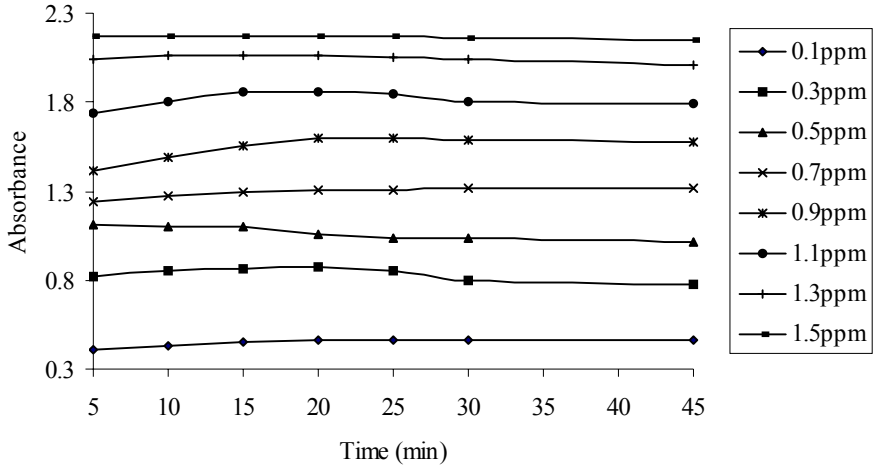


Fig. 4. Effect of sampling time.

detection (LOD). Naturally, the LOQ slightly crosses the lower limit of Beer's range and LOD is well below the lower limit of the Beer's range Khanmohmmadi *et al.,* 2007; Skoulika *et al.,* 2000; Basset *et al.,* 1986; Shama *et al.,* 2006). The upper limit of the Beer-Lambert range is determined by plotting the absorbance against concentration at the value of λ_{max} 395nm. Beyond this limit, the correlation results were really affected. Therefore, the measurement excluded above these limits to keep the relationship linear (Fig. 5).

Determination of Methyl Parathion in Water Samples

The distilled and tap water samples were fortified with the concentrations in the range of 0.1-1.5 ppm in methanol, which recovery data are given in table 2 and shown in figure 5. The fortified water samples were extracted with dichloromethane (DCM). The extracted samples were washed with 0.1M potassium carbonate solution to break any emulsion formed during the extraction and dried over anhydrous sodium sulphate. Finally dichloromethane was evaporated to dryness on a steam bath and the residue was dissolved in methanol and the amount was determined using the colorimetric procedures described in experimental details. Figure 4 shows that the formation of colour due to the coupling reaction of the methyl parathion samples with the reagents under instantaneous and stable for a reasonable period of time (sampling time 10 min) indicates its advantage with the results. This

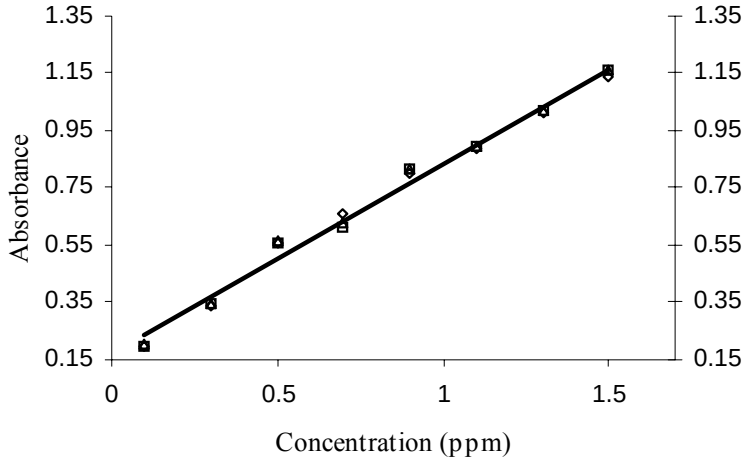


Fig. 5. Concentrations of MP Vs Absorbance.

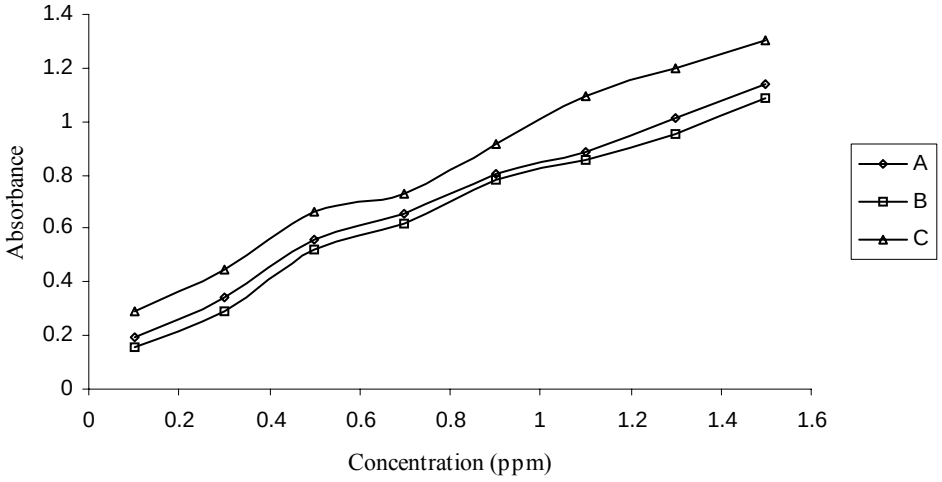


Fig. 6. Effect of temperature in absorbance.

procedure can be used to detect 0.1µg ml^{-1} to 1.5 µg ml^{-1} of methyl parathion. The correlation coefficient values obtained for both type of water samples (Tap and Distilled) were very close to unity suggesting that the absorbance depends upon the concentration of the methyl parathion. The values obtained for the relative standard deviation and percentage error suggest that these new procedures offer a good precision and accuracy. The results of the recoveries (Fig. 7) reveal that the amounts of methyl parathion determined spectrophotometrically**.** The data presented in table 1 suggests that the percentage of methyl parathion recovery from fortified water ranges from 95%-98.8%. The recovery tendency of the methyl parathion is mentioned in table indicate that their order in various environments. The recovery of MP in tape water was found to observe more than the distilled water as shown in figure 7.

Our observations supported the chemical degradation and detection procedure of methyl parathion in water samples via hydrolysis process. These observations suggest that the other ingredients present in these formulations do not interfere. The proposed methods are simple handling, low cost effective, rapid and sensitive. Therefore, this procedure can be adopted for a routine checkup in quality control, academics and laboratory.

CONCLUSIONS

The proposed spectrophotometric method is simple and cost effective. The sensitivity, simplicity, temperature independent stability (within range), recovery of the colored hydrolyzed samples are the advantages of this method. It is evident from the procedures that followed is simple, rapid and sensitive. More over, the methods do

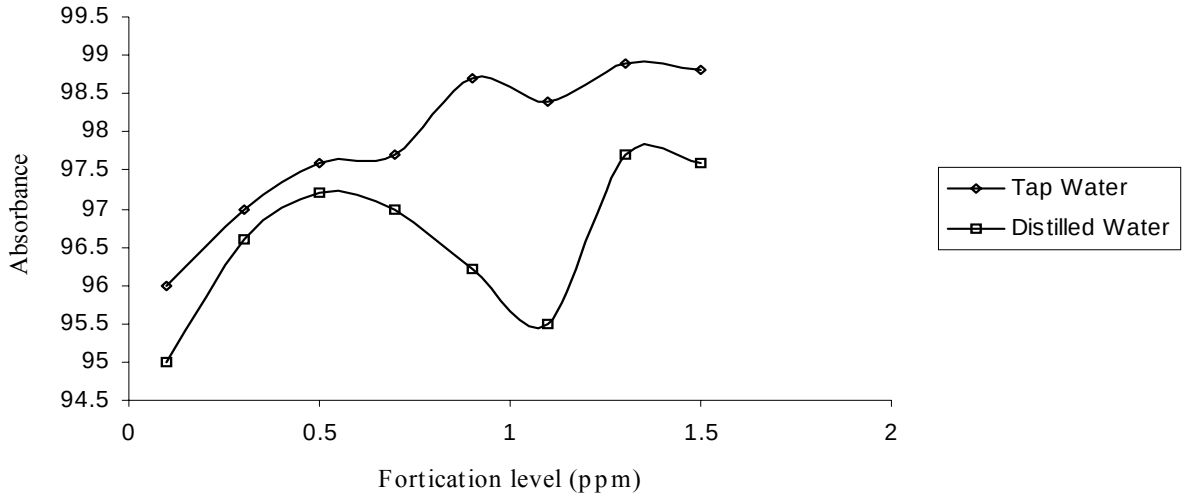


Fig. 7. Percentage Recovery of MP (Tap and Distilled water) Vs Fortification level.

Table. 2. For Suitability of Colorimetric Procedure

S.No.	Parameters of Methyl Parathion	Statistical Value
1.	λ_{max} (nm)	395
2.	Beer's law limit ($\mu g\ ml^{-1}$)	0.1ppm-1.5ppm
3.	Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	0.047
4.	Regression equation	Y = 0.6878+ 0.15476X
5.	Slope	0.15476
6.	Intercept	0.6878
7.	Correlation coefficient (r)	0.2128
8.	Standard deviation (SD)	0.043
9.	Relative standard deviation (RSD) %	5.3

not involve elaborate cleanup procedures as is required by the other methods. Therefore, the methods described here would serve as additional techniques for estimation of methyl parathion in fortified water samples.

Further work is continuing to quantify the concentration of methyl parathion in soil/water samples with the colorimetric procedures and comparison of results with sophisticated instruments like gas chromatography (GC).

ACKNOWLEDGEMENTS

The authors express their gratitude to Department of Science and Technology, Government of India to providing the financial support for this project.

REFERENCES

Lai, K., Stolowich, NJ. and Wild, JR. 1995. Characterization of P-S Bond Hydrolysis in Organophosphorothioate Pesticides by Organophosphorus Hydrolase. Archives of Biochemistry and Biophysics. 318:59-64.

Gallo, MA. and Lawryk, NJ. 1990. The Handbook of pesticides Toxicology. Academic Press, San Diego, CA. 2:917-1123.

Eichelberger, JW. and Lichtenberg, JJ. 1971. Persistence of pesticides in river water. Environment Science Technology. 5:541-544.

Gomaa, HM., Suffet, IN. and Faust, SD. 1969. Kinetics of hydrolysis of diazinon and diazoxon. Residue Reviews. 29:171-190.

Kuo, LY. and Perera, NM. 2000. Paraoxon and Parathion Hydrolysis by Aqueous Molybdenocene Dichloride (Cp_2MoCl_2): First Reported Pesticide Hydrolysis by an Organometallic Complex. American Chemical Society, Inorganic Chemistry. 39(10): 2103-2106.

Zheng, W. and Liu, W. 2002. Kinetics and mechanism of the hydrolysis of imidacloprid. Chinese Chemical Letters. 13 (12):1170-1173.

Serdar, CM., and Gibson, D. T. Enzymatic Hydrolysis of Organophosphates: Cloning and Expression of a

Parathion Hydrolase Gene from Pseudomonas diminuta. BioTechnology. 3:567-571.

Huang, X., Liu, J., Pi, Z. and Yu, Z. 2004. Qualitative and quantitative analysis of organophosphorus pesticides residues using temperature modulated SnO_2 gas sensor. Talanta. 64:538-545.

Jan, MR., Shah, J. and Khan, H. 2003. Investigation of new indirect spectrophotometric method for the determination of carbofuran in carbamate pesticides. Chemosphere**.** 52 (9):1623-1626.

Dannenberg, A. and Pehkonen, SO. 1998. Investigation of the Heterogeneously Catalyzed Hydrolysis of Organophosphorus Pesticides. Journal of Agricultural and Food Chemistry. 1(46):325-334.

Munnecke, DM. 1976. Enzymatic hydrolysis of organophosphate insecticides, a possible pesticide disposal method. Applcation of Environment Microbiology. 32(1):7-13.

Farooq, R., Feng-kai, L., Yu, W., Jian-jun, H., Zheng, X. and Shaukat, SF. 2004. Pressure hydrolysis for degradation of omethoate pesticide in water. 8: 221-226.

Khanmohammadi, M., Karimi, MA., Ghasemi, K., Jabbari, M. and Garmarudi, AB. 2007. Quantitative determination of Malathion in pesticide by modified attenuated total reflectance-Fourier transform infrared spectrometry applying genetic algorithm wavelength selection method. Talanta. 72:620-625.

Froey, K. 1982. Analytical profiles of drug substances. Academic Press, New York, NY, USA.

Skoulika, SG., Georgiou, CA. and Polissiou, MG. 2000. FT-Raman spectroscopy-analytical tool for routine analysis of diazinon pesticides formulations. Talanta. 51:599-604.

Basset, J., Denny, RC., Jeffery, GH. and Mendham, J. 1986. Text book of quantitative inorganic analysis, 4^{th} ed. Vol. 350.

Shama, SA., Amin, AE. and Omara, H. 2006. Colorimetric microdetermination of captopril in pure form and in pharmaceutical formulations, J. Quant. Spectroscopy & Radiative Transfer. 102:261-268.