CHAPTER 4

DISCUSSION AND CONCLUSIONS

4.1 Influence of Reaction Variables

For a sensitive determination using spectrophotometry as the indicator technique, it is necessary to obtain a product reaction with a high molar absorptivity. Previous experiments (58) indicate that the transformation takes place when addition of NaOH to the reagent solution is carried out. These results in a yellow coloration of the solution which can be observed by a bathochromic shift of the absorption maximal of methyl parathion (270nm) to that of 4-nitrophenol (400nm), with a molar absorptivity of 17,200 mol/l.cm. Absorption spectra of methyl parathion and its variation as a function of time are shown in Figure 4.1.

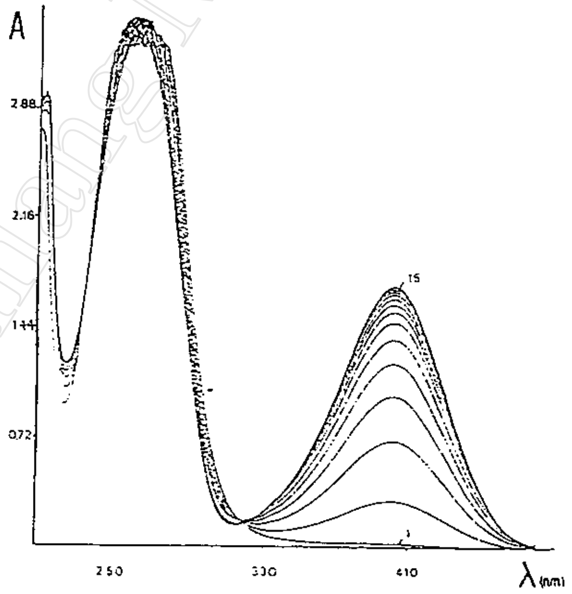

Figure 4.1 Normal absorption of the hydrolysis process of methyl parathion into 4-nitrophenol. [Methyl parathion]=26.32μg/ml; [NaOH]=0.1 M; T = 45°C. Curve1: 0 minute; Curve15: 45 minutes[58].

The hydrolysis of methyl parathion by alkaline alone to 4-nitrophenol is a slow process, depending upon the strength of the alkaline and the temperature. Ramakrishna *et al.*(35) found that the rate can be increased many hundred folds by substituting alkaline with a mixture of alkaline hydroxylamine with ethanol. The reaction rate is much faster at 25°C and almost instantaneous at 30°C. Preliminary studies of spectrometric determination of methyl parathion using alkaline hydroxylamine indicate that the maximum absorption of 4-nitrophenol as shown in Figure 2.3 is 403 nm and the reagent blank solution does not interfere in this region. Taking advantage of the above finding a procedure has been worked out for the spectrophotometric determination of methyl parathion via 4-nitrophenol liberated. The method is simple and quantitative and does not involve the use of drastic chemical procedures. These advantages procedures of batch spectrophotometric method proposed by Ramakrishna(35) was adopted for the development of novel FIA spectrophotometric method for determining methyl parathion in formulations and plant materials.

The alkaline hydrolysis of methyl parathion leading to its highly toxic and major metabolite 4-nitrophenol is shown is Figure 4.2.

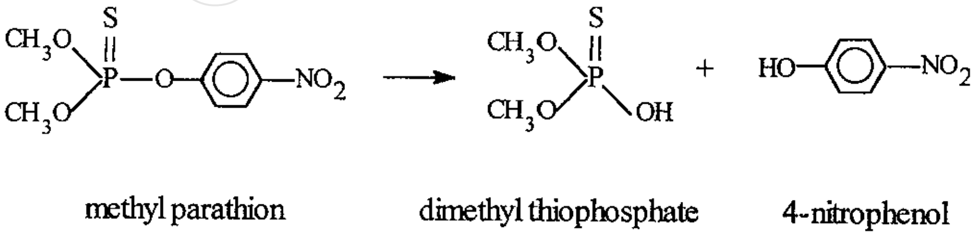

Figure 4.2 The hydrolysis reaction of methyl parathion to liberate 4-nitrophenol.

The optimization of the experimental condition starting with optimum wavelength of 4-nitrophenol liberated as shows in Table 2.2 and Figure 2.4 indicated that the maximum peak height is 410 nm and this wavelength was chosen for further FIA measurement.

The optimum concentrations of the reagents were chosen by changing their concentrations, the remaining variables being fixed. The optimum concentrations are those at which the sensitivity in the FIA measurement is maximal. The sodium hydroxide and hydroxylamine concentrations were varied over the range 1.5-3.5 M (Table 2.3 and Figure 2.5) and 0.6-2.2 M (Table 2.4 and Figure 2.6), respectively, to investigate their influence on the peak height. The sensitivity increased with the NaOH concentration up to 2.5 M. When the concentration of NaOH was greater than 2.5 M the sensitivity was slightly enhanced. From these studies, 2.5 M NaOH and 1.4 M hydroxylamine were suggested to establish the proposed FIA spectrophotometric method for methyl parathion determination.

Temperature has also an important influence on the peak height. Its effect was studied for the total reaction recording the peak height at different temperatures over the range 22.0-71.0°C at fixed sodium hydroxide and hydroxylamine concentrations (2.5 M and 1.4 M). Table 2.5 and Figure 2.7 indicate that temperature influences the peak height and sensitivity. The sensitivity increased with increasing temperature up to 50.5°C, a slight increase in stability was obtained when the temperature was higher than 50.5°C. Unfortunately, the bubbles frequently occur with increasing temperature, too. Thus a normal room temperature has been chosen to make the method.

The effect of the pump flow rate on peak height, sensitivity and sample throughput is presented in Table 2.6 and Figure 2.8-2.9. It was found that the sensitivity decreased while the sample throughput increased with increasing flow rate. The best result was obtained at the pump flow rate of 3.0 ml/min

The geometry of the mixing coil could affect the flow rate and hence, the sensitivity and sample throughput. The results of investigation for the optimum geometry of the first mixing coil R1 are shown in Tables 2.7-2.9 and Figures 2.10-2.12. The mixing coil R1 with the inner diameter of 0.04 inches, its length of 60.00 cm and its size of coiled mixing part of 0.80 cm were chosen as optimum. The geometry of the second mixing coil R2 was also studied in the same way. The results in Table 2.10-2.12 and Figure 2.13-2.15 indicate that the inner diameter of 0.02 cm, its length of 100.00 cm and its size of coiled mixing part of 0.50 cm are optimum.

Apart from the coiled mixing part, other types of mixing parts, namely single bead string reactors (s.b.s.r.), knitted reactors, straight reactors, and zigzag reactors were also tested. Optimum length of the s.b.s.r. R1 and R2 were investigated in order to obtain maximum sensitivity (Tables 2.13-2.14 and Figures 2.16-2.17). The lengths of 10.00 and 13.00 cm were chosen as optimum of the s.b.s.r. R1 and R2, respectively. The effects of various shapes of mixing part were also studied. The results in Table 2.15 and Figure 2.18 show that the s.b.s.r. mixing part is the optimum shape.

The effect of injection volume was investigated by varying a loop length of injection valve. The results in Table 2.16 and Figure 2.19 indicate the injection volume influences greatly the sensitivity and peak height. Both the sensitivity and peak height increase with increasing injection volume. Thus a volume of 500 μl was chosen as optimum injection volume with a reasonable of sample consumption.

4.2 Analytical Parameters

To obtain the calibration graph, different peak heights at the maximum wavelength of 410 nm have been recorded over the range 0.5-30 ppm at the optimized experimental conditions as shown in Table 2.17. The calibration graph was linear over the range of 0.5 to 15 ppm (Table 2.18 and Figure 2.20). Its equation is $Y = 14X - 5.2$ with a correlation coefficient of 0.9994 (Table 2.22 and Figures 2.21-2.22).

The theoretical detection limit defined as the minimum detectable quantity has resulted to be 0.7 ppm as shown in Table 2.20-2.21.

The accuracy and precision of the FI system have been determined with 12 replicates of 2-ppm methyl parathion. The result, expressed as the relative standard deviation, is 1.22%.

4.3 Interference Studies

There could be different ways in which other agricultural chemicals might interfere with the proposed FIA method. To evaluate the selectivity of the analytical method, a number of commonly occurring foreign species in vegetable samples were examined. The effect of the interference ions (calcium, magnesium, sodium, potassium, sulphate and phosphate) and other pesticides (malathion, methamidophos, monocrotophos and carbaryl) under the conditions described for analysis, were investigated. The results were obtained when applying the FIA method on the determination of 4-ppm methyl parathion in presence of one interference. Magnesium, potassium and carbaryl did not interfere up to 50-fold excess. Sulphate, phosphate and methamidophos did not interfere up to 100-fold excess. The tolerance limits for other interferences are given in Table 2.23.

4.4 Analysis of Methyl Parathion in Plant Material and Commercial Formulations

The highly toxic agricultural chemical methyl parathion is used in large quantities for insect control on cotton and other plants. It is part of numerous commercial formulations such as Folidol-E605 M50, Karlavin, Fonotox, Folez,etc.

These formulations contain methyl parathion as a 50% w/v active ingredient, and are presented as emulsifiable concentrates. Gupta *et al.* (33, 34) have used Folidol-E605 M50 as methyl parathion standard solutions. To demonstrate the applicability of the proposed method comparison with spectrophotometric and polarographic methods has been attempted. The commercial formulations were prepared as a 10-ppm of active ingredient and analyzed by these methods. The results are shown in Tables 3.2, 3.5, 3.7 and 3.9. It was found that most commercial formulations except Fonotox could be used as methyl parathion standard solution because the concentrations were found to be in good agreement with the certified values.

To evaluate the applicability of the proposed FIA method in plant materials, several different types of vegetable samples from Ton Payom Market in Chiang Mai were analyzed. The various vegetable samples were spiked with methyl parathion at several concentrations and analyzed as described. Typical quantitative results when applying the FIA, spectrophotometric and polarographic methods are shown in Tables 3.1, 3.4

and 3.6, respectively. Table 3.8 shows better recovery by the proposed method in comparison to the other methods.

4.5 Conclusions

The FIA approach using spectrophotometry as the detector technique has proved to be a useful alternative for the analysis of the highly toxic organophosphorus insecticide, methyl parathion. The alkaline hydrolysis carried out at room temperature with hydroxylamine and ethanol, which is in the samples, has permitted to establish an easy, rapid and safety method.

Optimized experimental conditions for methyl parathion determination are summarized in Table 4.1.

Table 4.1 Analytical characteristics for methyl parathion determination with alkaline hydroxylamine.

Analytical characteristics	Information
Wavelength	410 nm
NaOH concentration	2.5 M
H_3NOHCl concentration	1.4 M
Injection volume	500 µl
Pump flow rate	3.0 ml/min
Shape of mixing part	single bead string reactor
Length of mixing part R1 and R2	10 and 13 cm
Linear range of calibration curve	0.5-15 ppm
Slope of calibration curve	14 mV/ppm
Reproducibility	1.22%
Detection limit	0.7 ppm
Sample throughput	37 hr^{-1}

Double lines FI manifold, incorporating with two vertical settings of single bead string reactors equipped with a home-made debubbler for removing some gas bubbles liberated by hot weather, was developed for methyl parathion determination in commercial formulations and vegetable samples. The comparative results of the proposed FIA method with the spectrophotometric and polarographic methods indicate that the FIA method is more sensitive, rapid and convenient than the spectrophotometric method and more rapid and accurate than the polarographic method.

The proposed FIA method is thus suitable for use in the laboratories, which are not equipped with sophisticated instruments.