CHAPTER II
LITERATURE REVIEWS

This chapter contains two parts, including, the literature reviews of spectrophotometric analysis of pesticides and sample preparation for the analysis of pesticides.

2.1 Spectrophotometric analysis of pesticides

Several simple methods like spectrophotometry have been investigated for determination of carbamate pesticides. The methodologies were performed mostly by derivatization of the pesticides with appropriate labeling reagents to form clearly strong color solutions that can be easily applied for visible spectrophotometric detection.

Basically, the derivatization of pesticides is easily performed via diazotization reaction to form azo-dye compounds which provide high molar absorptivity (ε) with 3-4 orders of magnitude when compared to their native forms. There are a number of research works published on spectrophotometry for carbamate in different samples, some research works are given in the following paragraphs and summarized in Table 2.1.

Sastry et al. (1987) reported the use of *p*-aminophenol, *p*-dimetylphenylene diamine and 1-amino-2-naphthol-4-sulfonic acid as derivatizing agents for spectrophotometric determination of carbaryl and propoxur in insecticidal formulations, water and grain samples. The first two reagents can be used for derivatization both of carbaryl and propoxur, whereas the later reagent is only specific for carbaryl. High molar absorptivities of *p*-aminoplenol and *p*-dimetylphenylene-diamine derivatives were observed in range 1.0×10^4 – 1.4×10^4 L mol^{-1} cm^{-1} (λ_{max}= 600 nm) while the derivatives of 1-amino-2-naphthol-4-sulfonic acid gave 3.4×10^3 L mol^{-1} cm^{-1} (λ_{max}= 700 nm). However, chloroform (spectrum human carcinogen) was used for the extraction of the samples before the derivatization and also required long time for preparation.

Mathew et al. (1995) demonstrated a simple spectrophotometric method for the determination of carbaryl in soil and pesticides formulation, using 2-aminonaphthalene sulfonic acid (ANSA) as a derivatizing agent to form an azo-dye which has maximum absorbance at 490 nm ($\varepsilon = 1.86\times10^6$ L mol^{-1} cm^{-1}). This reagent easily formed derivative with only carbaryl, whereas the other carbamates could not form with ANSA reagent under the same derivatization condition. The present research used toxic organic solvent, i.e. trichloromethane (chloroform) for the extraction of samples before derivatization.

Venkateswarlu, Seshaiah (1995) used 4-aminoantipyrine as a derivatizing agent for determination of propoxur in water samples using sensitive spectrophotometric method. The method was based on the alkaline hydrolysis of propoxur and the resultant phenol was reacted with 2% w/v of 4-aminoantipyrine in the presence of an oxidizing agent, 8% w/v of potassium ferricyanide. The resultant colored dye was extracted into chloroform at pH 9.5 and the absorbance was measured at 472 nm. The molar absorptivity of the colored dye was 3.09×10^5 L mol^{-1} cm^{-1}. The colored dye was stable for more than 12 hour in chloroform, benzene and methyl isobutyl ketone but faded quickly in carbon tetrachloride.

Alvarez-Rodriguez et al. (1997) presented spectrophotometric determination of carbamate pesticides including carbaryl, bendiocarb, carbofuran, methiocarb, promecarb and propoxur. The pesticides were hydrolyzed in alkaline medium to 1-naphthol or phenolates and then coupled with diazotized trimethylaniline (TMA) in sodium dodecyl sulfate (SDS) micellar medium to form the azo dyes, the coupling reaction was completed after 15 min at room temperature. TMA reagent was stable at 5 °C for at least 48 hours, and at 20 °C the absorbance was reasonable constant for 10 hours. The reagent was therefore prepared every 48 hours and kept at low temperature. This procedure was applied to the determination of carbaryl in spiked tap, river, and pond water samples. The detection limits of the method were in the range of 0.2-2 mg L^{-1} and the repeatability was very good using borate buffer pH 9.5 for coupling and measuring steps. The molar absorptivities and LODs for the carbaryl sulfanilic acid derivatives were 1600 and 4000 L mol^{-1} cm^{-1} and 1.5 and 0.6 mg L^{-1} in non-micellar and micellar media, respectively.

Demirbas (1998) reported spectrophotometry for the determination of carbaryl and its hydrolysis product in soil and strawberry samples. The method was based on the coupling of 1-naphthol obtained from hydrolysis of carbaryl with diazotized sulphanilic acid in an alkaline medium to form *p*-(4-hydroxyl-1-naphtylaza benzenesulphonic acid which has a maximum absorbance at 475 nm. The method showed that the rate of hydrolysis of carbaryl was a function of pH and the solvent used, carbaryl had a great tendency to hydrolyze to 1-naphthol even in a highly diluted concentration of sodium hydroxide. The LODs of the proposed method was in the range 0.03-0.06 $\mu g\ L^{-1}$.

Manjubhashini et al. (2003) presented spectrophotometric determination of carbaryl in its formulations, water and food grains. The samples were extracted with chloroform before derivatization. After diazotization with 4-methoxyaniline and 4-aminoantipyrine, the products have high molar absorptivity about 5.33×10^4 L mol^{-1} cm^{-1} (λ_{max} = 490 nm) for 4-methoxyaniline and 2.51×10^4 L mol^{-1} cm^{-1} (λ_{max} = 480 nm) for 4-aminoantipyrine. The colored products were stable for 24 and 48 hours, respectively. Recoveries of carbaryl from fortified water and grain samples (0.8-4.8 mg L^{-1}) were in the range 95.0-98.0% for both reagents.

Kumar et al. (2005) reported the preparation of reagents for a sensitive spectrophotometric determination of carbaryl in environmental samples including its formulation, water, grains, soil and biological samples (blood and urine). Three reagents studied were 2,6-dibromo-4-methylaniline, 2,4,6-tribromoaniline and 2,6-dibromo-4-nitroaniline. The colored products were obtained as followed: purple with maximum absorption wavelength (λ_{max}) at 505 nm ($\varepsilon = 3.1\times10^4$ L mol^{-1} cm^{-1}), violet colored product (λ_{max} = 535 nm, $\varepsilon = 3.2\times10^4$ L mol^{-1} cm^{-1}), and pale-red product (λ_{max} = 492 nm, $\varepsilon = 4.0\times10^4$ L mol^{-1} cm^{-1}), respectively. These derivatives were instantaneous and stable over 36-48 hours. Limit of detections (LODs) of the proposed method was in the range 0.8-0.9 mg L^{-1}.

Santalad et al. (2008) used spectrophotometry for the determination of carbaryl residue in water and vegetables using acid-induced cloud-point extraction as preconcentration method. 2-napthylamine-1-sulfonic acid (ANSA) was used as derivatizing agent. Sodium dodecyl sulphate (SDS) was used as an extractant. The

derivatization was performed at room temperature by adding HCl, $NaNO_2$, ANSA and NaOH which corresponding to pH 11–12 for diazotization. The derivative has the maximum absorption wavelength at 480 nm ($\varepsilon = 3.0\times10^4$ L mol^{-1} cm^{-1}). The method shows good analytical features with detection limit of 50 μg L^{-1}. The linearity covered a wide range up to 7.0 mg L^{-1} and recoveries more than 85%.

Spectrophotometric technique cloud be simultaneous determine analytes in matrix samples. More techniques have been done in this mode, such as, partial less square technique, simultaneous equations technique, zero-crossing technique (Benamor, Aguerssif, 2008). There are many papers present simultaneous determination of analyte, especially metal ions, but there are a few papers present about simultaneous determination of pesticides. One research work on the analysis of pesticides using partial less square technique and two research works present simultaneous determination of metal ions using zero-crossing technique. The papers are shown in the following paragraph.

Khalaf et al. (1996) studied simultaneous kinetic determination of carbamate pesticides after derivatization with *p*-aminophenol using partial least squares (PLS). Propoxur, carbaryl, ethiofencarb and formetanate were used as the representative carbamates for this study in the fortified samples. The maximum absorption wavelengths for carbaryl and formetanate were 592 nm while propoxur and ethiofencarb were 604 and 630 nm, respectively. The results indicated that PLS allows spectrophotometry for the simultaneous determination of complex mixtures. However, good results only obtained for propoxur, carbaryl and ethiofencarb but formetanate cannot be determined accurately.

Elham, Hashem (2002) studied simultaneous determination of cadmium (II) and mercury (II) using zero-crossing technique. 4-(2-pyridylazo)-resorcinol was used as complexing agent. Cadmium (II) and mercury (II) in different ratios have been determined simultaneously. The zero-crossing technique is found suitable for the direct measurement of the first-derivative value at the specified wavelengths. Zero-crossing points of cadmium (II) and mercury (II) complexes are 510 nm and 522 nm, respectively. The LODs achieved were 21 μg L^{-1} of cadmium (II) and 34 μg L^{-1} of mercury (II).

Benamor et al. (2008) reported simultaneous determination of calcium and magnesium in pharmaceutical products by zero-crossing technique, first- and second-derivative spectrophotometric methods. The methods base on the colored complexes formed by calcium and magnesium with bromopyrogallol red in presence of Tween 80 as a surfactant. Zero-crossing of calcium and magnesium are 544.5 nm and 570 nm in first-derivative procedure and 574 nm and 531 nm in the second-derivative procedure, respectively. The LODs achieved were 0.0575 mg L^{-1} of calcium and 0.03 mg L^{-1} of magnesium.

Table 2.1 summaries the important items from the above mentioned literatures on the spectrophotometry for the analysis of pesticides.

Table 2.1 Literatures on determination of carbamate pesticides using spectrophotometry

Author (Year)	**Analyte**	**Sample**	**Reagent/ λ_{max}**	**Sample preparation**
Sastry et al. (1987)	carbaryl propoxur	water grains	*p*-aminophenol *p*-dimetylphenylene diamine *for both CBR and PPX/ 600 nm* 1-amino-2-naphthol-4-sulfonic acid *for only CBR / 700 nm*	LLE (chloroform)
Mathew et al. (1995)	carbaryl	soil	2-aminonaphtalene sulfonic acid (ANSA) /490 nm	LLE (chloroform)
Venkateswarlu et al. (1995)	propoxur	water	4-aminoantipyrine (4-AP) /472 nm	LLE (chloroform)
Khalaf et al. (1996)	propoxur carbaryl ethiofencarb formetanate	water	*p*-aminophenol / 592-636 nm	filtration

Table 2.1 Literatures on determination of carbamate pesticides using spectrophotometry (Cont.)

Author (Year)	**Analyte**	**Sample**	**Reagent/ λ_{max}**	**Sample preparation**
Alvarez-Rodriguez et al. (1997)	carbaryl bendiocarb carbofuran methiocarb promecarb propoxure	tap water pond water	Trimethylaniline (TMA)/ 510 nm	CPE (SDS)
Demirbas (1998)	carbaryl	soil strawberry	Diazotised sulphanilic acid/ 475 nm	LLE (chloroform)
Manjubhashini et al. (2003)	carbaryl	water fruit grains	4-methoxy aniline / 490 nm 4- aminoantipyrine / 480 nm	LLE (chloroform)
Kumar et al. (2005)	carbaryl	water grains soil biological-samples	2,6-dibromo-4-methylaniline / 505 nm 2,4,6-tribomoaniline / 535 nm 2,6-dibromo-4-nitroaniline/ 492 nm	LLE (chloroform)
Santalad et al. (2008)	carbaryl	water vegetables	2-naphthylamine-1-sulfonic acid (ANSA)/ 480 nm	CPE (SDS)

2.2 Sample preparation for the analysis of pesticides

General procedure for the analysis of pesticides in samples consists of sample preparation and instrumental analysis. Sample preparation procedure is based on two steps i.e. (i) extraction of the target analytes from samples and (ii) clean-up and/or preconcentration of the target analytes in the extracts obtained from step (i). In most cases for vegetable samples, both steps are needed. The extraction of the pesticides from vegetables, solid-liquid extraction (SLE) using organic solvents is the traditional method. The automated instrument based extraction technique, pressurized liquid extraction (PLE), was used to facilitate the extraction (Lee et al., 2008). While, liquid-liquid extraction (LLE) is a widely used method for clean-up and preconcentration of the target analytes. LLE has drawbacks of using large volume of toxic organic solvent and time consuming. Solid phase extraction (SPE) is an alternative technique for LLE. SPE is an effective method for clean-up and preconcentration but it has high cost of sorbents or cartridges and complex processes.

Recently, Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method was introduced as a simple, effective and inexpensive method to simultaneously extract and clean-up. QuEChERS has been used to remove complex matrices including sugars, lipids, organic acid, steroid, proteins, pigments and excess water from various samples (Anastassiade et al., 2003).

However, using spectrophotometry, the analytes are sometime detected with low sensitivity i.e. low signal. Thus, preconcentration step is required before spectrophotometric detection. LLE and SPE are commonly used for preconcentration, but they have some disadvantages as described previously. The use of surfactants as extractants, known as cloud-point extraction (CPE) or micelle-mediated extraction (MME) has gained increasingly acceptance as an alternative to the conventional extraction. CPE occurs at cloud-point temperature where the surfactant becomes cloudy, usually at a higher temperature than its critical temperature, resulting in a two phase separation involving the surfactant-rich phase (SRP) and the aqueous phase (AQP). Generally, analytes are present in the SRP which has a very small volume compared to the large AQP volume, thus they are concentrated with a high preconcentration factor ($F_C \approx V_{AQP}/V_{SRP}$). The SRP is then diluted with a minimum volume of organic solvent before analysis (Santalad et al., 2009).

Some research works on the analysis of pesticides using QuEChERS and CPE as the sample preparation methods are reported in the following paragraphs and summarized in Table 2.2.

Romeo-Gonzalez et al. (2008) reported the determination of 90 pesticides in fruit juice samples by ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS). QuEChERS was used for the extraction and the extracts were analyzed without any further clean-up step. The results were better than solid phase extraction procedure. For the extraction procedure, aliquot 10 mL of sample was pipetted to a polypropylene centrifuge tube and 10 mL of 1% of acetic acid in acetonitrile solution was added, and the mixture was vortexed for 1 min. Afterwards, 4 g of anhydrous magnesium sulfate and 1 g of sodium acetate were added and was shaken immediately for 1 min. After centrifugation, 1 mL of the supernatant was taken and diluted with 1 mL of water prior to UPLC–MS/MS analysis. The results showed that conventional QuEChERS procedure required a cleanup step with PSA (primary secondary amine) while the proposed buffer QuEChERS procedure did not use PSA.

Nguyen et al. (2008) presented a multi-residue method for the determination of 107 pesticides in cabbage and radish using QuEChERS. For sample preparation step, the grinded and homogenized sample was extracted with acetonitrile and then added $MgSO_4$ and NaCl, vortexed immediately and the extract was then centrifuged. The upper layer solution was transferred into a micro-centrifuge vial containing PSA and $MgSO_4$, vortexed and centrifuged again. The upper layer was then subjected to analyze by GC-MS. The recoveries for all the pesticides studied were 80-115%, and the relative standard deviation (RSD) lower than 15% in the concentration range of 0.03-0.36 mg kg^{-1}.

Lesueur et al. (2008) reported the modified QuEChERS method for analysis of 151 pesticides from conventional farming food stuff sample. Acetonitrile was added into homogenized samples and then magnesium sulphate, sodium chloride, sodium citrate and di-sodium hydrogen citrate sesquihydrate were added and vortexed immediately. The extract was adjusted to pH 5.0-5.5 using NaOH and centrifuged. The upper layer was transferred into a centrifuge tube containing PSA and $MgSO_4$.

The extract was centrifuged again, the upper layer was kept for analysis by GC-MS and HPLC-MS. The method was proved to be repeatable with RSD lower than 20%.

Lee et al. (2008) studied various sample preparation method for multi-residue pesticides in tobacco including the conventional liquid-liquid extraction (LLE), pressurized liquid extraction (PLE) and QuEChERS and then analysis by GC-MS. For QuEChERS method, the effect of sorbents, such as PSA, octadecylsilane (C_{18}) and graphitized carbon black (GCB), was investigated. C_{18} was able to remove co-extracted for waxes and fat with PSA and magnesium sulfate. GCB was used to remove carotenoids or chlorophyll with PSA and magnesium sulphate. The results indicated that QuEChERS using only PSA gave higher recoveries than conventional LLE and PLE methods.

Santalad et al. (2009) reported cloud-point extraction and reversed phase high-performance liquid chromatography (HPLC) for determination of carbamate pesticides including, methomyl, propoxur, carbofuran, carbaryl, isoprocarb and promecarb in fruits. Triton X-114 (TX-114) was used as an extractant in CPE of carbamate pesticides. The SRP was analyzed by reversed phase HPLC and detected with ultraviolet detection at 270 nm. The mobile phase composed of methanol and acetic acid. The limits of detection were in the range of 0.1-1.0 mg kg^{-1}. The recoveries of spiked fruit sample were 80-107%.

Chen et al. (2009) used a cloud-point extraction for preconcentration of carbofuran derivative prior to analysis by HPLC. Carbofuran was hydrolyzed with NaOH to form 2,3-dihydro-2,2-dimethyl-7-benzofuran (BF) and was then coupled with 4-aminoantipyrine (AP) in the presence of potassium ferricyanide ($K_3Fe(CN)_6$) to generate red colored derivative (BFAP) which has the maximum absorbance at 530 nm. The cloud-point extraction methodology using Triton X-100 (TX-100) as an extractant was applied as a preconcentration step prior to HPLC, the SRP containing BFAF was then analyzed in visible region. In this method, carbofuran could be determined with recoveries ranging from 80.4% to 84.5%.

Table 2.2 summaries the important items from the above mentioned literatures on the sample preparation (QuEChERS and CPE) for the analysis of pesticides.

Table 2.2 Literatures on sample preparation

Author (year)	Analyte	Sample	Sample preparation	Analytical technique
Romeo-Gonzalez et al. (2008)	90 pesticides	fruit juices	QuEChERS: Sample were mixed with 1% acetic acid in acetonitrile, magnesium sulphate and sodium acetate were added. The mixture was centrifuged and supernatant was diluted before analysis.	UHPLC/ tandem-MS
Nguyen et al. (2008)	107 pesticides	cabbage radish	QuEChERS: Sample was extracted with CH_3CN and then $MgSO_4$ and NaCl were added, shaken and centrifuged, the supernatant was added with PSA and $MgSO_4$, vortexed and centrifuged again, the upper layer was analyzed.	GC-MS
Lesueur et al. (2008)	151 pesticides	conventional farming food stuff	QuEChERS: Sample were extracted with CH_3CN and then $MgSO_4$, NaCl, sodium citrate and di-sodium hydrogen citrate were added and centrifuged. The upper layer was mixed with PSA and $MgSO_4$, centrifuged again, the upper layer was analyzed.	GC-MS HPLC-MS

Table 2.2 Literatures on sample preparation (Cont.)

Author (year)	Analyte	Sample	Sample preparation	Analytical technique
Lee et al. (2008)	multi-residue pesticides	tobacco	Liquid-Liquid Extraction (LLE) Pressurized Liquid Extraction (PLE) QuEChERS: C18, PSA and $MgSO_4$ were used to remove co-extracted for wax and fat, GCB with PSA and $MgSO_4$ were used to remove carotenoids or chlorophyll.	GC-MS
Santalad et al. (2008)	carbaryl	water vegetables	SLE: Homogenized vegetable sample were extracted using $MeOH/H_2O$ (50/50 v/v). The extract was then subjected to CPE. CPE: The extract was mixed with SDS and HCl. The SRP was diluted with water before spectrometric analysis.	Spectrophotometry

Table 2.2 Literatures on sample preparation (Cont.)

Author (year)	Analyte	Sample	Sample preparation	Analytical technique
Santalad et al. (2009)	methomyl propoxur carbofuran carbaryl isoprocarb promecarb	apple pear guava	SLE: Homogenized sample was extracted with CH_3CN. The extract was then preconcentrated by CPE. CPE: The extract was added with TX-114 and NaCl solution and then at 45 °C for 20 min and then centrifuged. The SRP was diluted with methanol before analysis.	HPLC-PDA
Chen et al. (2009)	carbofuran	rice	CPE: Rice powder was placed in centrifuge tube and then TX-100 solution was added. The tube was kept in the ultrasonic bath. The supernatant was filtered and analyzed by HPLC.	HPLC-MS and HPLC-vis (530 nm)