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## **APPENDICES**

## **APPENDIX A**

### **Experimental data**

## 1. Derivatization of CBR using DMA

**Table 1A** Effect of concentration of reagents for derivatization of CBR by DMA

Reagent	Concentration (mmol L <sup>-1</sup> )	Absorbance, mean (n=3)
DMA	0.2, 0.4, 0.6, 0.8, 1.0	0.011, 0.017, 0.024, 0.024, 0.025
NaNO <sub>2</sub>	0.1, 0.2, 0.4, 0.6, 0.8, 1.0	0.248, 0.244, 0.171, 0.072, 0.040, 0.05
HCl	2.0, 4.0, 6.0, 8.0, 10.0	0.289, 0.288, 0.285, 0.283, 0.271
NaOH	2.0, 4.0, 6.0, 8.0, 10.0, 12.0	0.076, 0.190, 0.243, 0.329, 0.357, 0.359

**Table 2A** Stability of CBR derivative

Time (min)	Absorbance, mean (n=3)
10, 20, 30, 50, 70, 90, 110, 130	0.211, 0.212, 0.214, 0.216, 0.221, 0.228, 0.238, 0.231

## 2. Derivatization of CBR and CBF using AP

**Table 3A** Effect of concentration of reagents on the absorbance of CBR and CBF derivatives

Reagent	Concentration (mmol L <sup>-1</sup> )	Absorbance, mean (n=3)
<b>AP:</b>		
CBR	1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0	0.033, 0.060, 0.084, 0.121, 0.122, 0.124, 0.125
CBF	1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0	0.039, 0.065, 0.096, 0.120, 0.134, 0.134, 0.136
<b>K<sub>3</sub>Fe(CN)<sub>6</sub>:</b>		
CBR	2.4, 4.8, 7.2, 9.6, 12, 14.4, 16.8	0.03, 0.05, 0.096, 0.123, 0.119, 0.114, 0.112
CBF	2.4, 4.8, 7.2, 9.6, 12, 14.4, 16.8	0.021, 0.06, 0.103, 0.135, 0.138, 0.131, 0.128
<b>pH:</b>		
CBR	8, 9.5, 11.4, 12.3, 12.8, 13, 14	0.01, 0.178, 0.19, 0.172, 0.088, 0.05, 0.045
CBF	8, 9.5, 11.4, 12.3, 12.8, 13, 14	0.015, 0.223, 0.22, 0.134, 0.099, 0.038, 0.030
<b>Buffer:</b>		
CBR	0.0, 1.0, 3.0, 5.0, 7.0	0.030, 0.075, 0.100, 0.120, 0.130
CBF	0.0, 1.0, 3.0, 5.0, 7.0	0.039, 0.085, 0.121, 0.139, 0.141



**Table 4A** Stability of CBR and CBF derivatives

Time (min)	Absorbance, mean (n=3)
<b>CBR derivative</b> 10, 20, 30, 50, 70, 90, 110, 130	0.129, 0.125, 0.125, 0.122, 0.126, 0.127, 0.129, 0.124
<b>CBF derivative</b> 10, 20, 30, 50, 70, 90, 110, 130	0.140, 0.142, 0.145, 0.148, 0.140, 0.142, 0.147, 0.140

### 3. Cloud-point extraction

**Table 5A** Effect of concentration of reagents on the extraction efficiency of CBR and CBF

Reagent/ condition	Concentration (%w/v)	Absorbance, mean (n=3)
<b>TritonX-114:</b> CBR-DMA CBR-AP CBF-AP	0.5, 1.0, 1.5, 2.0, 2.5	0.510, 0.712, 0.830, 0.800, 0.661 0.402, 0.500, 0.604, 0.520, 0.427 0.350, 0.440, 0.511, 0.470, 0.452
<b>NaCl:</b> CBR-DMA CBR-AP CBF-AP	0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 2.0, 3.0	0.402, 0.535, 0.659, 0.790, 0.810, 0.818, 0.820, 0.826, 0.830 0.300, 0.350, 0.601, 0.650, 0.660, 0.670, 0.682, 0.671, 0.682 0.401, 0.452, 0.470, 0.543, 0.550, 0.854, 0.610, 0.611, 0.631
<b>Equilibration temperature:</b> CBR-DMA CBR-AP CBF-AP	25, 35, 45, 55 °C	0.651, 0.672, 0.701, 0.713 0.452, 0.502, 0.524, 0.522 0.351, 0.459, 0.492, 0.490
<b>Equilibration time:</b> CBR-DMA CBR-AP CBF-AP	5, 10, 15, 20, 25, 30 min	0.325, 0.692, 0.780, 0.786, 0.788, 0.789 0.300, 0.430, 0.550, 0.532, 0.551, 0.570 0.330, 0.410, 0.472, 0.480, 0.480, 0.49



4. Ratio of mobile phase for HPLC

**Table 6A** Ratio of mobile phase for the separation of CBR and CBF by HPLC

MeOH/H <sub>2</sub> O (v/v)	Retention time (min), mean (n=3)	
	CBF	CBR
30/70	4.50	5.95
50/50	3.10	4.01
70/30	1.91	2.50
80/20	1.32	1.51

## **APPENDIX B**

### **Calculations**



### 1. Concentration of pesticides ( $\text{mg L}^{-1}$ ) in spiked sample

The sample preparation steps consisted of extraction by QuEChERS technique and preconcentration by CPE. In QuEChERS technique, sample blended (15.0000 g) was spiked with 150  $\mu\text{L}$  pesticide ( $100 \text{ mg L}^{-1}$  stock solution) and then extracted with 25.00 mL acetonitrile (exceed water was removed by salts). The organic phase solution 20.00 mL was evaporated to eliminate acetonitrile and then the solution was diluted with water and adjusted volume to 10.00 mL. In CPE procedure, the sample solution from QuEChERS (10.00 mL) was preconcentrated and analyzed.

The final concentration in  $\text{mg kg}^{-1}$  of the pesticide in sample was calculated as described in the following paragraphs.

Calculation of the amount of pesticides in sample:

Pesticide 150  $\mu\text{L}$  from stock  $100 \text{ mg L}^{-1}$  contains pesticide

$$\frac{0.15 \times 100}{1000} = 0.015 \text{ mg}$$

Then, sample (15.0000 g) contains pesticide 0.015 mg

$$\text{Sample } 1,000 \text{ g contains pesticide } \frac{0.015 \times 1000}{15} = 1.0 \text{ mg}$$

i.e. The sample contains pesticide  $1.0 \text{ mg kg}^{-1}$

The final concentration in  $\text{mg L}^{-1}$  of the pesticides before preconcentrated by CPE was calculated as follow:

After extraction step, 25.00 mL organic solvent contains pesticide 0.015 mg, then 20.00 mL organic solvent contains pesticide  $\frac{0.015 \times 20}{25} = 0.012 \text{ mg}$ . The organic solvent (20.00 mL) was evaporated and adjusted to 10.00 mL, then final concentration of pesticide was calculated as follow:

Solution 10 mL contains pesticide 0.012 mg

$$\text{Solution } 1000 \text{ mL contains pesticide } \frac{0.012 \times 1000}{10} = 1.2 \text{ mg}$$

Then, final concentration of pesticide before preconcentrate by CPE is  $1.2 \text{ mg L}^{-1}$ .

## 2. Percentage recovery

The percentage recovery can be obtained via the following equation:

$$\%Recovery = \frac{C_f - C_i}{C_s} \times 100$$

Where:  $C_f$  = Concentration of pesticide found in sample after spiking pesticides standard ( $\text{mg L}^{-1}$ )

$C_i$  = Initial concentration of pesticide found in real sample ( $\text{mg L}^{-1}$ )

$C_s$  = Spiked concentration of pesticide standard which added into real sample ( $\text{mg L}^{-1}$ )

For example;

The calibration equation for the determination of pesticide in cucumber is

$$Y = 0.121X + 0.003, R^2 = 0.999 \text{ (see Table 4.9)}$$

If CBR was spiked at  $1.2 \text{ mg L}^{-1}$  ( $C_s = 1.2 \text{ mg L}^{-1}$ ) and then was analyzed by spectrophotometry, giving absorbance of 0.1311, the  $C_f$  can be calculated as follow:

$$0.1311 = 0.121X + 0.003$$

$$X = 1.06, \text{ then } C_f = 1.06 \text{ mg L}^{-1}$$

If cucumber was not contaminated with CBR, then  $C_i = 0.00 \text{ mg L}^{-1}$

The recovery can be calculated as follow:

$$\begin{aligned} \%Recovery &= \frac{1.06 - 0.00}{1.2} \times 100 \\ &= 88.3\% \end{aligned}$$

Therefore, percent recovery is 88.3 %.

## 3. Comparing individual differences

A *t*-test is used to compare one set of measurements with another to decide whether or not they are different. We arbitrarily set the probability level at 95% for concluding that two means differ from each other. If there is less than a 95% probability, then we will conclude that they do not differ from each other. In this study, *t*-test was applied for the case of two different methods to make single

measurements on several different samples. The typical example for calculation is described below.

Example; from Table 4.13, comparison of recovery that obtained from the propose spectrophotometric technique and HPLC, the data are shown in Table 1B.

Table 1B *t*-test for CBR

Number of pair of data	Recovery (%)		Difference ( <i>d<sub>i</sub></i> )
	Spectrophotometry	HPLC	
1	88.89	86.39	2.5
2	92.06	91.67	0.39
3	95.86	91.39	4.47
4	81.67	85.83	-4.16
5	82.77	85.37	-2.6
			<i>d</i> = +0.12

The *t* value can be calculated using the following equations (Danial C. Harris, 1998)

$$t_{calculated} = \frac{\bar{d}}{s_d} \sqrt{n}$$

where,

$$s_d = \sqrt{\frac{\sum (d_i - \bar{d})^2}{n - 1}}$$

The quantity *d* is the average difference between methods (spectrophotometry and HPLC) and n is number of pairs of data (five in this case). For the data in Table 1B, *s<sub>d</sub>* is

$$\begin{aligned} s_d &= \sqrt{\frac{(2.5 - 0.12)^2 + (0.39 - 0.12)^2 + (4.47 - 0.12)^2 + ((-4.16) - 0.12)^2 + ((-2.6) - 0.12)^2}{5 - 1}} \\ &= \sqrt{12.59} = 3.55 \end{aligned}$$

then,

$$t_{calculated} = \frac{0.12}{3.55} \sqrt{5} = 0.08$$





$t_{calculated}$  is compared with  $t_{table}$  for  $n-1$  degrees of freedom . If  $t_{calculated}$  is greater than  $t_{table}$  at the 95% confidence level, the two results are considered to be different. The  $t_{table}$  are summarized in Table 2B.

For  $n-1 = 5-1= 4$  degree of freedoms in Table 2B for 95% confidence level ( $p=0.05$ ) is 2.78.

Then  $t_{calculated} (0.08) < t_{table} (2.78)$ , the difference is not significant.

**Table 2B** The  $t$ -test distribution

Degree of freedom	Probability, p			
	0.1	0.05	0.01	0.001
1	6.31	12.71	63.66	636.62
2	2.92	4.30	9.93	31.60
3	2.35	3.18	5.84	12.92
4	2.13	2.78	4.60	8.61
5	2.02	2.57	4.03	6.87
5	1.94	2.45	3.71	5.96
6	1.89	2.37	3.50	5.41
7	1.86	2.31	3.36	5.04
8	1.83	2.26	3.25	4.78
9	1.81	2.23	3.17	4.59
10	1.80	2.20	3.11	4.44
11	1.78	2.18	3.06	4.32
12	1.77	2.16	3.01	4.22
13	1.76	2.14	2.98	4.14
14	1.75	2.13	2.95	4.07
15	1.75	2.12	2.92	4.02
20	1.70	2.04	2.75	3.65
30	1.68	2.02	2.70	3.55
infinity	1.65	1.96	2.58	3.29

**4. Standard deviation; SD**

The SD can be obtained via the following equation:

$$SD = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$$

$\bar{x}$  = Mean of the analytical results (n)

**5. Relative standard deviation; RSD**

The %RSD can be obtained via the following equation:

$$\%RSD = \frac{SD}{\bar{x}} \times 100$$

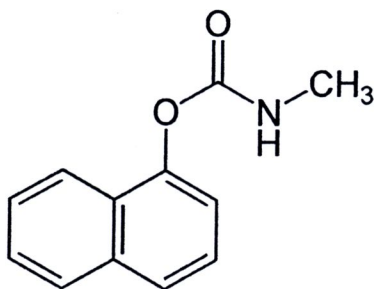
## **APPENDIX C**

### **Toxicities of CBR and CBF**



## 1. Carbaryl

**IUPAC name:** 1-naphthyl-N-methylcarbamate



**Figure 1C** Structure of CBR

**Acute toxicity:** Carbaryl is moderately to very toxic. It can produce adverse effects in humans by skin contact, inhalation, or ingestion. The symptoms of acute toxicity are typical of the other carbamates. Direct contact of the skin or eyes with moderate levels of this pesticide can cause burns. Inhalation or ingestion of very large amounts can be toxic to the nervous and respiratory systems resulting in nausea, stomach cramps, diarrhea, and excessive salivation. Other symptoms at high doses include sweating, blurring of vision, in coordination, and convulsions. The only documented fatality from carbaryl was through intentional ingestion. The oral  $LD_{50}$  of carbaryl ranges from  $250 \text{ mg kg}^{-1}$  to  $850 \text{ mg kg}^{-1}$  in rats, and from  $100 \text{ mg kg}^{-1}$  to  $650 \text{ mg kg}^{-1}$  in mice. The inhalation  $LC_{50}$  in rats is greater than  $200 \text{ mg L}^{-1}$ . Low doses can cause minor skin and eye irritation in rabbits, a species in which carbaryl's dermal  $LD_{50}$  has been measured at greater than  $2,000 \text{ mg kg}^{-1}$ .

**Chronic toxicity:** Not Available

**Reproductive effects:** No reproductive or fetal effects were observed during a longer study of rats fed high doses of carbaryl.

**Teratogenic effects:** The evidence for teratogenic effects due to chronic exposure is minimal in test animals. Birth defects in rabbit and guinea pig offspring occurred only at dosage levels that were highly toxic to the mother.

**Mutagenic effects:** Carbaryl has been shown to affect cell division and chromosomes in rats. However, numerous studies indicate that carbaryl poses only a slight mutagenic risk. There is a possibility that carbaryl may react in the human

stomach to form a more mutagenic compound, but this has not been demonstrated. In sum, the evidence suggests that carbaryl is unlikely to be mutagenic to humans.

**Carcinogenic effects:** Technical-grade carbaryl has not caused tumors in long-term and lifetime studies of mice and rats. Rats were administered high daily doses of the pesticide for 2 years, and mice for 18 months, with no signs of carcinogenicity. While N-nitrosocarbaryl, a possible by-product, has been shown to be carcinogenic in rats at high doses, this product has not been detected. Thus, the evidence indicates that carbaryl is unlikely to be carcinogenic to humans.

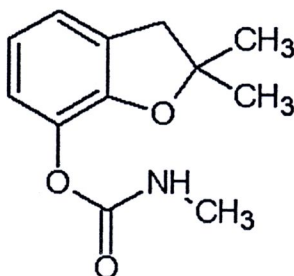
**Organ toxicity:** Ingestion of carbaryl affects the lungs, kidneys, and liver. Inhalation will also affect the lungs. Nerve damage can occur after administration of high doses for 50 days in rats and pigs. Several studies indicate that carbaryl can affect the immune system in animals and insects. Male volunteers who consumed low doses of carbaryl for 6 weeks did not show symptoms, but tests indicate slight changes in their body chemistry. A 2-year study with rats revealed no effects at or below a dose of 10 mg/kg/day.

**Fate in humans and animals:** Most animals, including humans, readily break down carbaryl and rapidly excrete it in the urine and feces. Workers occupationally exposed by inhalation to carbaryl dust excreted 74% of the inhaled dose in the urine in the form of a breakdown product. The metabolism of up to 85% of carbaryl occurs within 24 hours after administration.



## 2. Carbofuran

**IUPAC name:** 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate



**Figure 2C** Structure of CBF

**Acute toxicity:** Carbofuran is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption. As with other carbamate compounds, carbofuran's cholinesterase-inhibiting effect is short-term and reversible. Symptoms of carbofuran poisoning include: nausea, vomiting, abdominal cramps, sweating, diarrhea, excessive salivation, weakness, imbalance, blurring of vision, breathing difficulty, increased blood pressure, and incontinence. Death may result at high doses from respiratory system failure associated with carbofuran exposure. Complete recovery from an acute poisoning by carbofuran, with no long-term health effects, is possible if exposure ceases and the victim has time to regain their normal level of cholinesterase and to recover from symptoms. The oral  $LD_{50}$  is 5 to 13  $mg\ kg^{-1}$  in rats, 2  $mg\ kg^{-1}$  in mice, and 19  $mg\ kg^{-1}$  in dogs. The dermal  $LD_{50}$  is  $>1000\ mg\ kg^{-1}$  in rabbits. The  $LC_{50}$  (4-hour) for inhalation of carbofuran is 0.043 to 0.053  $mg\ L^{-1}$  in guinea pigs.

**Chronic toxicity:** Rats given very high doses (5  $mg/kg/day$ ) for two years showed decreases in weight. Similar tests with mice gave the same results. Prolonged or repeated exposure to carbofuran may cause the same effects as an acute exposure.

**Reproductive effects:** Consuming high doses over long periods of time caused damage to testes in dogs, but carbofuran did not have any reproductive effects on rats or mice. Available studies indicate carbofuran is unlikely to cause reproductive effects in humans at expected exposure levels.



**Teratogenic effects:** Studies indicate carbofuran is not teratogenic. No significant teratogenic effects have been found in offspring of rats given carbofuran (3 mg/kg/day) on days 5 to 19 of gestation. No effects were found in offspring of mice given as much as 1 mg/kg/day throughout gestation. In rabbits, up to 1 mg/kg/day on days 6 to 18 of gestation was not teratogenic.

**Mutagenic effects:** Weak or no mutagenic effects have been reported in animals and bacteria. Carbofuran is most likely nonmutagenic.

**Carcinogenic effects:** Data from animal studies indicate that carbofuran does not pose a risk of cancer to humans.

**Organ toxicity:** Carbofuran causes cholinesterase inhibition in both humans and animals, affecting nervous system function.

**Fate in humans and animals:** Carbofuran is poorly absorbed through the skin. It is metabolized in the liver and eventually excreted in the urine. The half-life in the body is from 6 to 12 hours. Less than 1% of a dose will be excreted in a mother's milk. It does not accumulate in tissue.

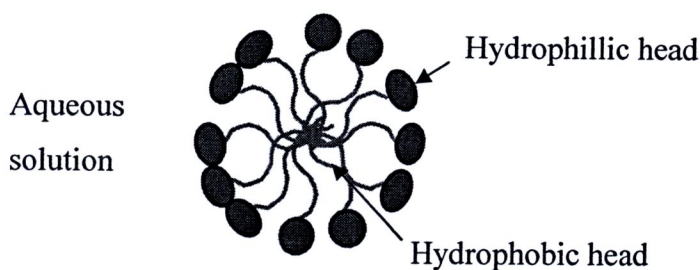


## **APPENDIX D**

### **Surfactant**

## Surfactant

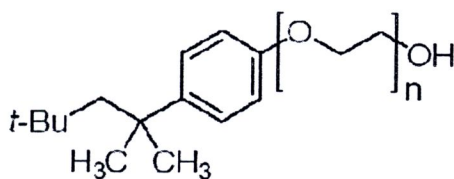
The term surfactant is a blend of “surface acting agent”. Surfactants are usually organic compounds that are amphipathic, meaning they contain both hydrophobic groups (their “tails”) and hydrophilic groups (their “heads”). Therefore, they are soluble in both organic solvents and water. Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution into aggregates. Some of these aggregates are known as micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their heads form an outer shell that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain favorable contact with oil. Surfactants are also often classified into four primary groups; anionic, cationic, non-ionic, and zwitterionic (dual charge) surfactant.



**Figure 1D** Scheme of micelle formed by surfactants in aqueous solution



Triton X-114 as one type of non-ionic surfactant. CMC of triton X-114 as  $0.2 \text{ mmol L}^{-1}$  at  $20\text{-}25^\circ\text{C}$ , structure of triton X-114 is shown below.



**Figure 2D** Structure of Triton X-114;  $n = 7\text{-}8$

## **APPENDIX E**

### **Maximum residue limits of CBR and CBF in vegetable samples**



## 1. The Japan Food Chemical Research Foundation (JFCRF)

The JFCRF settles the regulation for maximum residue limits (MRLs) of CBR and CBF in vegetables. The MRLs of vegetable samples are summarized in Table 1E.

**Table 1E** MRLs of CBR and CBF in vegetables; JFCRF

Vegetable sample	MRL (mg kg <sup>-1</sup> )	
	Carbaryl (CBR)	Carbofuran (CBF)
Cucumber	3.0	0.5
Cabbage	1.0	0.5
Kale	10.0	0.5
Yard long bean	1.0	0.2
Mustard	1.0	0.5

## 2. ASEAN cooperation in food

The ASEAN cooperation in food settles the regulation for MRLs of CBR and CBF for vegetables as summarized in Table 2E that adopted by 22<sup>nd</sup> ASEAN Ministers on Agriculture and Forestry (22<sup>nd</sup> AMAF), 26-27 October 2000, Phnom Penh.

**Table 2E** MRLs of CBR and CBF in vegetables; ASEAN

No.	Crops	Codex MRLs (mg kg <sup>-1</sup> )	ASEAN MRLs (mg kg <sup>-1</sup> )
<b>Carbaryl (CBR)</b>			
1	Cabbages, head	5.0	5.0
2	Melons, except water melon	3.0	3.0
3	Cucumber	3.0	3.0
4	Leafy vegetables	10.0	10.0
5	Peppers	5.0	5.0
6	Okra	10.0	10.0
7	Tomato	5.0	5.0
8	Sweet corn	1.0	1.0
9	Peas	5.0	5.0
10	Common beans	5.0	5.0
11	Radish	2.0	2.0
12	Carrot	2.0	2.0
13	Potato	0.2	0.2
14	Asparagus	10.0	10.0
<b>Carbofuran (CBF)</b>			
1	Onion, bulb	0.1	0.1
2	Cabbages, head	0.5	0.5
3	Cauliflower	0.2	0.2
4	Lettuce, head	0.1	0.1
5	Egg plant	0.1	0.1
6	Tomato	0.1	0.1
7	Sweet corn (kernels)	0.1	0.1
8	Carrot	0.5	0.5
9	Potato	0.5	0.5
10	Maize	0.1	0.1

### 3. Thai Agricultural Commodity and Food Standard (TACFS 9002-2008)

The TACFS settles MRLs of CBR and CBF for vegetables as summarized in Table 3E.

**Table 3E** MRLs of CBR and CBF in vegetables; TACFS

No	Crops	MRLs (mg kg <sup>-1</sup> )
<b>Carbaryl (CBR)</b>		
1	Cucumber but water melon	3.0
2	Melon	1.0
3	Cabbage vegetables	5.0
4	Peanut	2.0
5	Chilli	0.5
<b>Carbofuran (CBF)</b>		
1	Cucumber but water melon	0.3
2	Melon	0.1
3	Yard long bean	0.1
4	Peanut	0.1
5	Pepper	1.0

## **APPENDIX F**

### **Simultaneous determination techniques by spectrophotometry**



### 1. Simultaneous equations technique

The spectrophotometry can determine two substances by making measurement at two wavelengths. Two dissimilar chromophores must necessarily have different power of light absorption at some point or points in the spectrum. If, therefore, measurements are made on each solution at two such points, a pair of simultaneous equations may be obtained from which the two unknown concentrations may be determined. First, select two points on the wavelength scale where the ratios of the molar absorptivities are maxima; that is,  $(\epsilon_1/\epsilon_2)_{\lambda_1}$  and  $(\epsilon_2/\epsilon_1)_{\lambda_2}$  are maxima for the system illustrated in Figure 1F. Next, calculate the molar absorptivity for each component using a particular set of sample containers and spectrophotometer to be employed in the analyses. Since absorbance is directly proportional to the product of molar absorptivity and concentration, if the light path remains constant, it is possible to set up two simultaneous equations:

$$A_{\lambda_1} = C_1(\epsilon_1)_{\lambda_1} + C_2(\epsilon_2)_{\lambda_1}$$

$$A_{\lambda_2} = C_1(\epsilon_1)_{\lambda_2} + C_2(\epsilon_2)_{\lambda_2}$$

And solve for the concentration of each component:

$$C_1 = \frac{(\epsilon_2)_{\lambda_2} A_{\lambda_1} - (\epsilon_2)_{\lambda_1} A_{\lambda_2}}{(\epsilon_1)_{\lambda_1} (\epsilon_2)_{\lambda_2} - (\epsilon_2)_{\lambda_1} (\epsilon_1)_{\lambda_2}}$$

$$C_2 = \frac{(\epsilon_1)_{\lambda_1} A_{\lambda_2} - (\epsilon_1)_{\lambda_2} A_{\lambda_1}}{(\epsilon_1)_{\lambda_1} (\epsilon_2)_{\lambda_2} - (\epsilon_2)_{\lambda_1} (\epsilon_1)_{\lambda_2}}$$

When,  $A_{\lambda_1}$  and  $A_{\lambda_2}$  are absorbance of species at wavelength 1<sup>st</sup> and 2<sup>nd</sup>

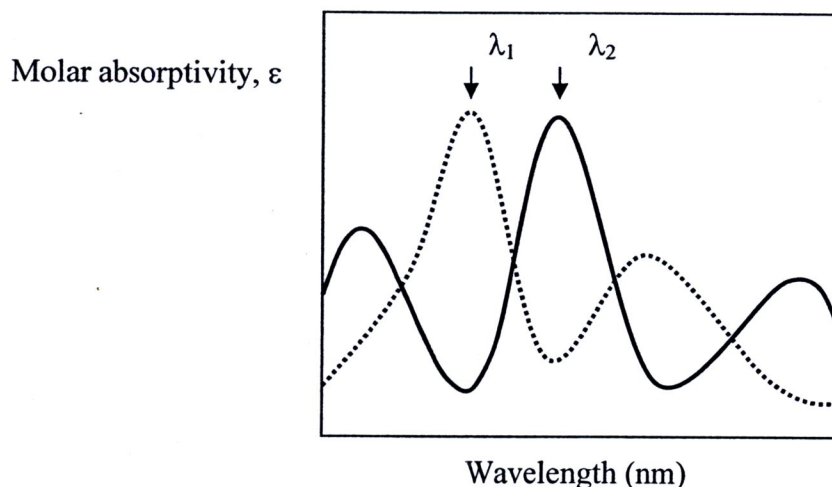
$C_1$  and  $C_2$  are concentrations of species 1<sup>st</sup> and 2<sup>nd</sup>

$\epsilon_1$  and  $\epsilon_2$  are molar absorptivities of species 1<sup>st</sup> and 2<sup>nd</sup>, respectively

The same procedure may be applied, in principle to multi component systems. However, the accuracy is poor unless the spectra are quite discrete, which is seldom true of electronic spectra. Simultaneous determinations rest on the assumption that



the substance concerned contribute additively to the total absorbance at an analytical wavelength. This assumption should be tested with known mixtures of the test materials.



**Figure 1F** Simultaneous spectrophotometric analysis of a two-component system . Selection of analytical wavelengths indicated by arrows  
(Willard et al., 1974)

## 2. Zero-crossing technique

The first-derivative spectrophotometry using zero-crossing technique for simultaneous determination of analytes in the binary mixture based on the measurements of the absolute value of the derivative spectrum of the mixture at wavelength (abscissa value) where the sensitivity of one component of the mixture goes to zero. At this wavelength, the intensity is directly proportional to the other component (Elham et al., 2002).

## RESEARCH PUBLICATIONS

1. Suksant Karnsa-ard and Supalax Srijaranai. **Determination of carbaryl in vegetables using spectrometry coupled to cloud point extraction.** Poster presentation at the 35<sup>th</sup> Congress on Science and Technology of Thailand (STT35); 2009 October 15<sup>th</sup> – 17<sup>th</sup>; The Tide Resort, Bangsaen Beach, Chonburi, Thailand.
2. Suksant Karnsa-ard and Supalax Srijaranai. **Cloud point extraction coupled to spectrophotometry for the determination of carbaryl in vegetables.** Oral presentation at the 11<sup>th</sup> Graduate Research Conference 2010; 2010 February 12<sup>th</sup>; Khon Kaen University, Khon Kaen, Thailand.

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