Journal of Chromatographic Science 2012;50:523–530
doi:10.1093/chromsci/bms043 Advance Access publication April 23, 2012

Article

Role of Different Salts on Cloud-Point Extraction of Isoprocarb and Promecarb Insecticides Followed by High-Performance Liquid Chromatography

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Received 18 May 2011; revised 11 August 2011

The influence of salt additive on cloud point extraction (CPE) of isoprocarb and promecarb insecticides is described. Types of salt (Na_2CO_3, $CaCl_2$, $MgSO_4$, Na_2SO_4, $NaHCO_3$ and NaCl) and concentrations were studied. The extracted target compounds were analyzed using reversed phase high-performance liquid chromatography. Among the salts studied, Na_2CO_3 was found to be the most effective salt for salting out of both insecticides, resulting in high extraction efficiency (>95%) and high enrichment factor of up to 18 compared to extraction without preconcentration. The optimum CPE conditions were 1.5% (*w*/*v*) Triton X-114, 3.0% (*w*/*v*) Na_2CO_3, and 20-min equilibration at 45°C. Under the selected conditions, the linear range of 0.05 to 3.0 mg/L was found for both analytes. The limits of detection for isoprocarb and promecarb were 10 and 20 μg/L, respectively. High intra-day ($n = 9$) and inter-day ($n = 3 \times 4$ days) precisions with relative standard deviations <1% and <8% were obtained for retention time and peak area, respectively. The proposed method was successfully applied for the residue analysis of target compounds in beverages (i.e., fruit juice, vegetable juice and wine samples), which provided high recoveries (>80%, on average) for spiked samples at three levels (0.05, 0.10 and 0.50 mg/L).

Introduction

Cloud-point extraction (CPE) is a popular surfactant-based method for preconcentration of various compounds in different samples before instrumental analysis, e.g., high-performance liquid chromatography (HPLC) (1–8). An interesting aspect of CPE is that the analytes are extracted by surfactants from a large bulk aqueous solution and consequently concentrated in a lower volume of the surfactant-rich phase. CPE has been recognized as an easy and solvent-free extraction method compared to conventional organic liquid–liquid extraction. A process of the CPE phenomenon has been described previously (1–7). In brief, at a certain temperature (i.e., higher than the cloud point temperature), an aqueous solution of non-ionic surfactant becomes cloudy due to the decreasing solubility of the surfactant in water and phase separation occurs to form a surfactant-dilute aqueous phase (AQ) and a surfactant-rich phase (SRP). The SRP contains a high concentration of surfactant, whereas the AQ contains surfactant monomers or micelles [with concentration near their critical micelle concentration (CMC)]. The SRP usually has a much smaller volume than the AQ, and thus a high preconcentration factor can be obtained. The solute molecules are distributed between the two phases under CPE conditions, depending on their hydrophobicity. For this purpose, non-ionic surfactants; e.g., Triton X-100 (1, 2), Triton X-114 (3–5), and Genapol X-080 (6, 7) have been widely used as the solubilization media for a wide variety of solutes. Generally, CPE can be carried out using proper concentration and temperature conditions without additives, but in many cases, the addition of salts (e.g., NaCl and Na_2SO_4) can facilitate phase separation and improve extraction efficiency (1–7). The phenomenon is based on salting out and salting in of cations and anions that are present in the aqueous micellar solution (5, 9, 10). Thus, the appropriate selection of salts to induce CPE has been investigated to improve the capability of the extracting target analytes (9, 11–18).

In our previous work (19), we investigated CPE using Triton X-114 and NaCl to improve the sensitivity for the analysis of six carbamate insecticides. The method gave high extraction efficiency and resulted in low limits of detection (LODs) at the μg/L level for the four studied analytes (i.e., methomyl, carbaryl, carbofuran and propoxur). However, it was not sensitive for isoprocarb and promecarb. Thus, a sensitive and selective method was further developed to improve detections of these insecticides for ensuring safety under the maximum residue limits (MRLs) in variety of food sample matrices.

In the present work, the effect of a variety of salt additives (e.g., Na_2CO_3, $CaCl_2$, $NaHCO_3$, $MgSO_4$, Na_2SO_4, and NaCl) was intensively investigated to improve capability of CPE for isoprocarb and promecarb. The effect of ethanol on CPE performance was also investigated because it is present at approximately 12% (*v*/*v*) in the wine samples. The proposed method has been applied to residue analysis in fruit juices, vegetable juices and local wines.

Experimental

Chemicals and reagents

All reagents were of analytical grade. Isoprocarb (IPC) and promecarb (PMC) were purchased from Sigma-Aldrich (Seelze, Germany). The standard stock solutions (1,000 mg/L) of carbamate insecticides were prepared by dissolving appropriate amounts in methanol. Triton X-114 was purchased from Acros (Morris Plains, NJ) and used without further purification, and a stock solution (25%, *w*/*v*) was prepared by dissolving appropriate amount in deionized water. Sodium chloride (NaCl) was obtained from Ajax Finechem (New South Wales, Australia). Sodium carbonate anhydrous (Na_2CO_3), sodium sulfate (Na_2SO_4), sodium bicarbonate ($NaHCO_3$), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), calcium chloride ($CaCl_2$), NaOH

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pellets and concentrated HCl from Carlo Erba (Val de Reuil, France) were also used. 2,4-Dimethoxyaniline (DMA) was purchased from Fluka (Japan). Methanol (HPLC grade) was purchased from Lab Scan Asia Co. (Bangkok, Thailand). Acetic acid (glacial, obtained from Carlo Erba, Val de Reuil, France) was used for preparation of the HPLC mobile phase.

Instrumentation

Chromatographic separation was performed on a Waters Tiger LC System (Waters, Milford, MA) with an ultraviolet (UV) detector, and the Clarity software (Waters) was used for data acquisition. A Waters liquid chromatograph equipped with a Waters 2996 photodiode array detector (PDA) and Empower software was also used to confirm retention behavior and absorption spectra of the target compounds. A Waters Symmetry C18 column (3.9 × 150 mm, 5 μm) was used. A thermostat water bath (ISOTEMP 228, Thermo Fisher Scientific, Waltham, MA) was used to implement cloud point extraction. A centrifuge (Biomed Group Co., Bangkok, Thailand) was used for complete phase separation.

Sample preparation

The studied samples included commercial fruit juices, vegetable juices and local wines produced in Thailand and available in the local markets of Khon Kaen. The samples were kept at 5°C until analysis. Before CPE, samples were filtered through filter paper (Whatman #1). Fruit and vegetable juices were directly analyzed after filtration, whereas wine samples were diluted 10 times with water before analysis. To investigate recovery assay, the spiked sample solutions (final concentrations of 0.05, 0.10 and 0.50 mg/L after dilution) were prepared by adding appropriate amounts of insecticide reference standards. These diluted solutions were then extracted with the CPE procedure described in the following.

CPE procedure

An aliquot of standard or diluted sample solution and specified salt solution were mixed so that the final salt concentration was 3.0% (*w*/*v*). The pHs of solutions were in the range 7–12, depending on each salt studied (see detail in the Section Effect of salt additives). After that, 1.50% (*w*/*v*) Triton X-114 was added, resulting in a cloudy solution. This solution was then placed in a thermostatted bath at 45°C for 20 min and then centrifuged at 3,500 rpm for 20 min. After the separation of two phases, the aqueous phase (upper part) was removed by syringe and the surfactant-rich phase (SRP) present at the bottom of the solution was kept in an ice bath for 10 min to obtain the viscous SRP. The SRP was then diluted with methanol (300 μL) before analysis by HPLC. Volumes of aqueous phase and SRP were measured using syringe and microsyringe to calculate phase volume ratio.

Chromatographic conditions

The SRP solution was analyzed by reversed-phase HPLC conditions as determined by our previous work (19): gradient mobile phase of methanol and 0.1% (*v*/*v*) acetic acid, pH 5, at a flow rate of 0.7 mL/min, with a gradient profile of 60% (*v*/*v*) methanol ramped to 70% (0–5 min) and then ramped to 80% methanol (6–10 min) and continuously held for 5 min. To wash the excess surfactant off the column, 100% methanol was used for 5 min before the next run. The injection sample volume was 20 μL. The separation was carried out at 25°C and detection at 270 nm.

Identification and confirmation of hydrolyzed carbamate compounds

To identify and confirm the formation of the hydrolyzed form of carbamates (i.e., the phenolate compounds) after the CPE, two methods were used, as follows: (i) absorption spectra and (ii) diazotization reaction. For diazotization reaction, a coupling reagent solution of DMA (1.5 mmol/L) containing $NaNO_2$ (7 mmol/L) and HCl (50 mmol/L) was added into the studied carbamate solutions after addition of Na_2CO_3. An intense orange-red colored solution with an absorption maximum around 470 nm was obtained.

Results and Discussion

Effect of salt additives

Effect of salt type and pH of solution

To study the effect of salts on the extraction of the target analytes, different salts of mono- and divalent ions, including Na_2CO_3, $CaCl_2$, $MgSO_4$, Na_2SO_4, $NaHCO_3$ and NaCl (3.0%, *w*/*v*, for each salt studied in 10 mL of diluted sample solution) were tested and compared to solutions without salt addition. The concentrations of these salts were calculated to be approximately 283 mmol/L Na_2CO_3, 270 mmol/L $CaCl_2$, 250 mmol/L $MgSO_4$, 211 mmol/L Na_2SO_4, 357 mmol/L $NaHCO_3$ and 513 mmol/L NaCl. The results (Figure 1) demonstrate that Na_2CO_3 and $CaCl_2$ provided higher responses (i.e., peak area) for both analytes (approximately 6–8 times for Na_2CO_3 and 3 times for $CaCl_2$) when compared to those obtained from the

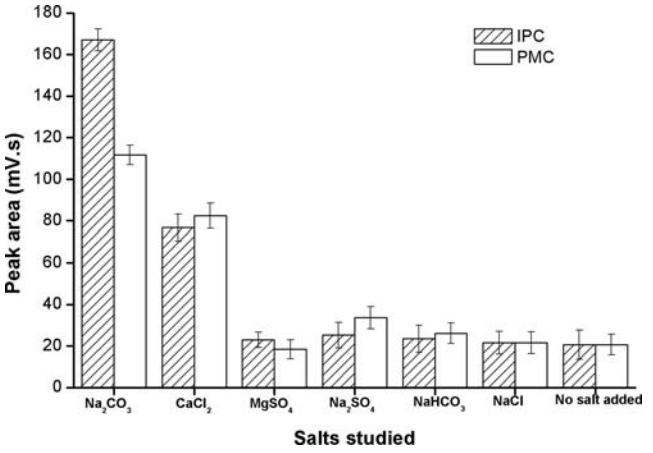


Figure 1. Effect of the addition of various salts on the peak area of IPC and PMC compared to that of without salt addition. Conditions: 1.0 mg L^{-1} of each insecticide, 1.5% (*w*/*v*) Triton X-114, 3.0% (*w*/*v*) salt, 20-min equilibrated at 45 °C, 20-min centrifuged at 3500 rpm.

other salts or those without salt addition. The effect of the studied salts on analytical signal of the target compounds can be ordered as follows:

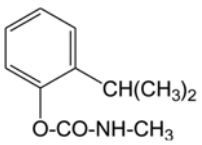
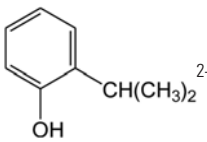
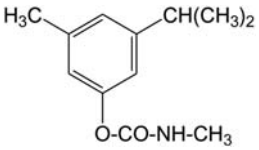
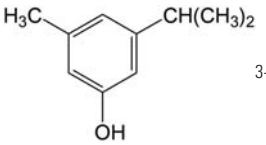
Na_2CO_3 >> $CaCl_2$ >> $MgSO_4 \approx Na_2SO_4 \approx NaHCO_3 \approx$ NaCl ≈ no salt addition. Except for $MgSO_4$, the relative salting-out power observed for the different salt additives seems to be in agreement with the ionic strength of each salt. Their ionic strengths were ordered to be: $MgSO_4$ (1.0) > Na_2CO_3 (0.85) > $CaCl_2$ (0.81) > Na_2SO_4 (0.63) > NaCl (0.51) > $NaHCO_3$ (0.36). It has been reported that the addition of salts to the surfactant micellar solution can increase or decrease its cloud point temperature (CPT) (12); Na_2SO_4 and Na_2CO_3 were found to be more effective than NaCl in decreasing CPT. It has also been reported (20) that the ionic strength of the solution can alter the CPT and facilitate the separation of two phases by altering the density of the bulk aqueous phase. As results show, salts derived from mono- and divalent ion pairs like $2Na^+/ CO_3^{2-}$ and $Ca^{2+}/ 2Cl^-$ gave higher peak responses than monovalent ions (e.g., Na^+/Cl^- and Na^+/HCO_3^-) or divalent ions (e.g., Mg^{2+}/SO_4^{2-}). An exception was the salt $2Na^+/ SO_4^{2-}$. Considering Na_2CO_3 versus $NaHCO_3$, $CaCl_2$ versus NaCl, and $MgSO_4$ versus Na_2SO_4, the ability to enhance the signal of the target compounds is $CO_3^{2-} > HCO_3^-$, $Ca^{2+} > Na^+$, and $Mg^{2+} > Na^+$, respectively. Purkait *et al.* (16) reported that the salting-out effect is more pronounced for divalent salt ($CaCl_2$) than monovalent salt (NaCl). In fact, both cations and anions affect the CPE (21, 22), and it has also been reported that the effect of cations on salting-out was smaller than anions (17). However, the capability of salts to enhance the extraction efficiency cannot completely be concluded by ionic strength because it is also dependent on other parameters such as experimental conditions and counter ions. (23).

To extend the investigation, the effect of pH of the solution after the addition of salts was also considered because the carbamate insecticides can be hydrolyzed to the corresponding phenolic compounds under basic conditions (24–26). In this study, salts studied can be classified into two groups, as follows:

(1) The salts that gave neutral solutions (pH = 7), i.e., Na_2SO_4, NaCl and $MgSO_4$; and
(2) The salts that gave alkaline solutions (pH > 7), i.e., $NaHCO_3$ (pH 8.8), $CaCl_2$ (pH 10.8) and Na_2CO_3 (pH 11.5).

It was found (results not shown) that the solutions at approximately pH 7 did not affect the retention times of both analytes. Meanwhile, longer elution times of both compounds were observed in basic conditions, especially with Na_2CO_3 and $CaCl_2$ salt additives. However, Na_2CO_3 provided the highest peak responses of both insecticides. It is assumed that changes in retention time of the analytes result from conversion of the original molecule (Species I) to the phenolate form (Species II). The expected phenolate structure and some characteristics of the studied compounds are shown in Table I. To prove the assumption, CPE conditions (without salt addition) were further studied by adding 1 mol/L NaOH and 1 mol/L HCl with an appropriate amount into the solutions instead adding electrolyte salts to provide the desired different pHs (pH 5.0–11.0) before CPE. The chromatograms and the absorption spectra of the species are illustrated in Figures 2 and 3, respectively. It was shown that when the solution pH was lower than 9.0,

Table I
Physical Properties and Some Characteristics of the Parent Studied Carbamates (Species I) and their Hydrolyzed Forms (Species II)*

Name	Structure	CAS no.	Mw (g/mol)	Aqueous solubility (mg/L)	log $K_{ow}^{\dagger}$
Isoprocarb I (IPC – I)		2631-40-5	193.25	270‡	2.37
Isoprocarb II (IPC – II)	 2-Isopropylphenol	88-69-7	136.19	NA/insoluble	2.97
Promecarb I (PMC – I)		2631-37-0	207.27	91‡	3.18
Promecarb II (PMC – II)	 3-Isopropyl-5-methylphenol	NA	150.22	NA/insoluble	3.52§

*NA: not available.
†The logarithm of octanol–water partition coefficient (log P_{ow} or log K_{ow}) accessed from http://www.srcinc.org/what-we-do/databaseforms.aspx?id=385.
‡Santalad *et al.* (19).
§Obtained by calculating from 5-isopropyl-2-methylphenol (CAS: 499-75-2) and 2-isopropyl-5-methylphenol (CAS: 89-83-8).

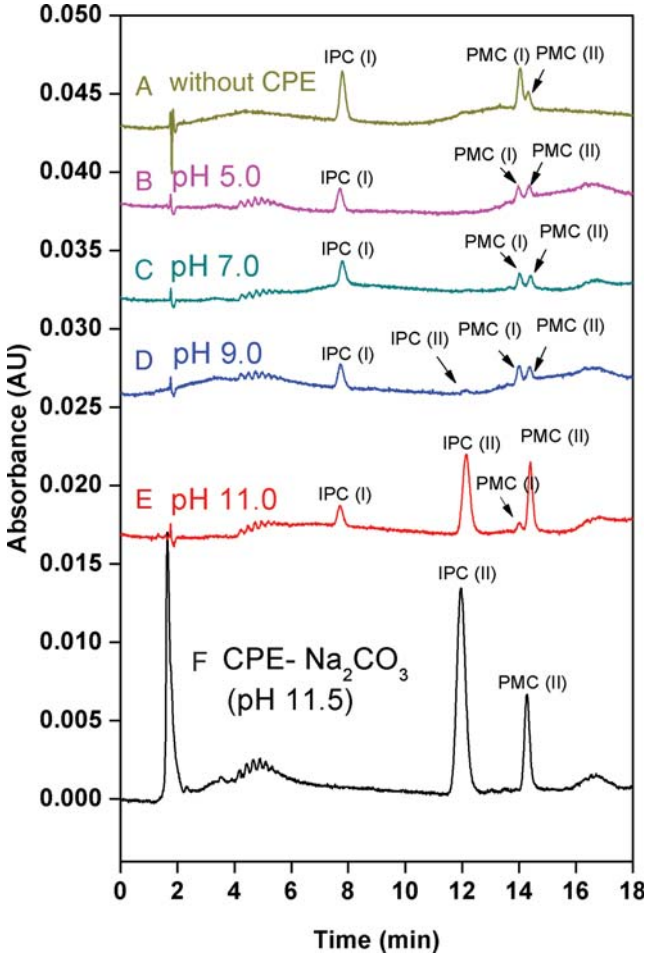


Figure 2. Chromatograms of IPC − I/II and PMC − I/II obtained from (A) direct injection (without CPE), (B) – (E) different pHs in CPE without salt addition, and (F) CPE with addition of Na_2CO_3. Other conditions: (A) 10 mg L^{-1} each pesticide; (B) – (F) 1.0 mg L^{-1}, CPE conditions as described in Figure 1.

isoprocarb (IPC − I) was not significantly influenced by pH changes. The same retention times (Figures 2B–2D) and similar spectra (Figures 3B–3D) as those obtained without CPE (Figures 2A and 3A) were observed. In contrast, promecarb was strongly affected by the pH changes in all studied ranges in which two species (PMC − I and PMC − II) were found with different retention times and spectra. At high pH, i.e., pH >11.0, the results indicate that both PMC − I and IPC − I were completely hydrolyzed to PMC − II and IPC − II, respectively. As shown in Figure 2E, small peaks of native forms (Species I) and larger peaks of their phenolic forms (Species II) were detected. Meanwhile, both peaks of IPC − II and PMC − II in the presence of Na_2CO_3 revealed complete hydrolysis of the parent insecticides. In addition, the results were consistent with the absorption spectra obtained from PDA measurements (Figure 3F). As a result, phenolate species of isoprocarb (IPC − II) was detected with a longer retention time (t_R shifted > 4 min) than the native compound (IPC − I), whereas PMC − I and its phenolate (PMC − II) showed a slight change in retention time (t_R shifted < 0.5 min).

This behavior can be explained by considering the logarithm of octanol − water partition coefficient (log K_{ow} or log P_{ow}), which correlates with chromatographic retention factors (k') (27) and refers to the hydrophobicity of the analytes. According to the estimated log K_{ow} of each species in Table I, it is clear that phenolate species of both analytes exhibit a higher log K_{ow} value than their native compounds particularly, IPC − I; however, its log K_{ow} is lower than that of promecarb. In conclusion:

(i) Phenolate species are more hydrophobic than their native forms; and

(ii) Promecarb (both native PMC − I and phenolate PMC − II species) has higher hydrophobicity than IPC − I/ − II.

Therefore, the retention times were observed to correlate with their log K_{ow} as follows: IPC − I <<< IPC − II << PMC − I < PMC − II. Because the phenolate species has less steric hindrance along with higher hydrophobicity, more penetration of phenolate forms into micellar aggregates, resulting in higher extraction efficiency are expected, especially for PMC − II. The extraction efficiency (%E) can be calculated as the following equation (28):

$$\%E = \left[1 - \frac{1}{(R_v + 1)} \frac{C_w}{C_{SRP}}\right] \times 100 \qquad (1)$$

where R_v is the volume ratio of SRP to aqueous phase; C_w is the concentration of analytes in aqueous phase (original solution before CPE); and C_{SRP} is the concentration of target analytes in SRP, which was quantified using the calibration graph obtained from original solutions (without CPE). The %E of the whole CPE method using Na_2CO_3 was 95.0 for IPC − II and 96.5 for PMC − II, whereas the CPE using NaCl provided %E of 82 and 67 for IPC − I and PMC − I, respectively. This indicates that the extraction of both insecticides in the phenol forms is more effective than the extraction of parent compounds. As the results show in Figure 3, the bathochromic phenomena was observed with a shift toward longer wavelength of phenolate species (270–280 nm), compared to the native forms (261–265 nm). To confirm the presence of phenolate species, the diazotization reaction of DMA was chosen in this study. DMA in the presence of sodium nitrite ($NaNO_2$) and HCl can easily react with hydrolyzed carbamates to form an intense orange-red color solution in basic medium with an absorption maximum around 470 nm. This study concluded that Na_2CO_3 as salt additive in CPE not only promotes the salting-out ability, but also alters the analyte form, which can enhance extraction efficiency of the studied compounds.

Effect of Na_2CO_3 concentration

Because Na_2CO_3 gave a higher % E for both analytes than the other studied salts, the effect of Na_2CO_3 concentration in the range 0–7.0 % (*w/v*) was investigated (data not shown). It was clear that the peak area of both analytes increased with an increase in salt concentration up to 3.0% (*w/v*) by a factor of 4–6 compared to those without salt (0%). Above 7.0% (*w/v*), a strong salting-out effect produced an SRP that became milky and moved to the surface of the solution, making it difficult to measure and to handle the volume. Therefore, 3.0% (*w/v*) Na_2CO_3 was selected as the optimum content.

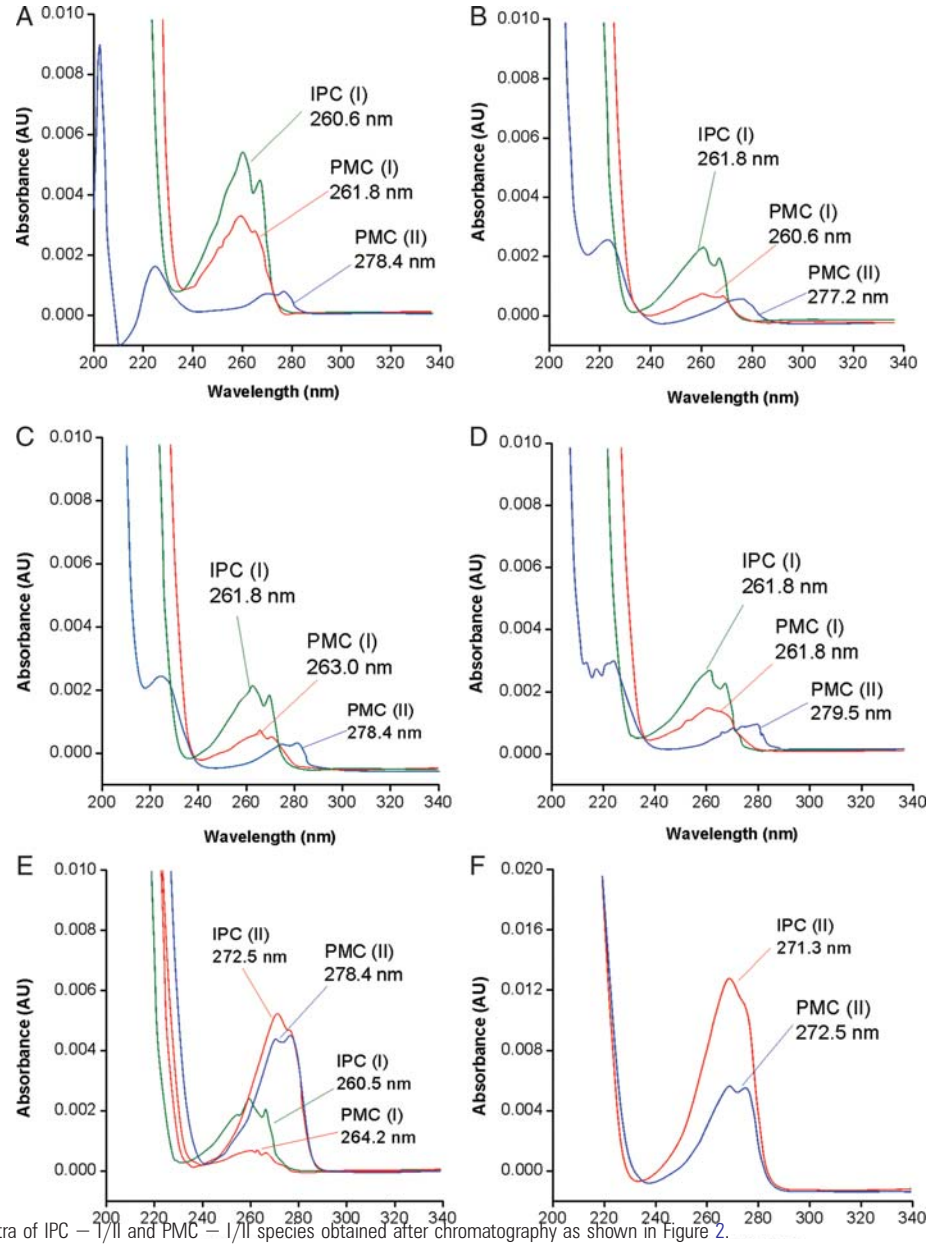


Figure 3. Absorption spectra of IPC − I/II and PMC − I/II species obtained after chromatography as shown in Figure 2.

Analytical performance and method validation

Analytical parameters such as linearity, LOD and limit of quantitation (LOQ) of the standards obtained from CPE induced by Na_2CO_3 (CPE − Na_2CO_3), were compared with those from experiments without preconcentration. These parameters are summarized in Table II. The lowest concentration of the linear range of calibration graph obtained from the method using Na_2CO_3 was 0.05 mg/L, which is 10 times lower than the analysis without preconcentration. Under the optimum CPE conditions, the phase volume ratio of AQ and SRP was approximately 17. The enrichment factor (EF) was calculated as the ratio of the slope of the calibration graph obtained using CPE and without preconcentration (Table II). The EF was 18.1 and 8.5 for isoprocarb and promecarb, respectively. Although PMC − II has relatively higher %E, IPC − II has higher absorbance by approximately 2.5 times at 270 nm (see spectra in Figure 3F). Thus, higher response (sensitivity) for IPC − II, resulting in

Table II
Calibration and Analytical Parameters of the Studied Insecticides Obtained from the Proposed CPE method and Without Preconcentration

Insecticide	Linearity (mg/L)	Linear equation (n = 3, 5 levels)	R^2	LOD (mg/L)	LOQ (mg/L)
IPC-II	0.05–3.0 (0.50–10)*	Y = 184.5X − 0.87 (Y = 10.2X + 0.13)	0.9999 (0.9995)	0.01 (0.3)	0.05 (0.5)
PMC-II	0.05–3.0 (1.0–10)	Y = 115.8X + 1.06 (Y = 13.6X + 0.70)	0.9998 (0.9987)	0.02 (0.5)	0.05 (1.0)

*The values in parentheses are obtained without preconcentration.

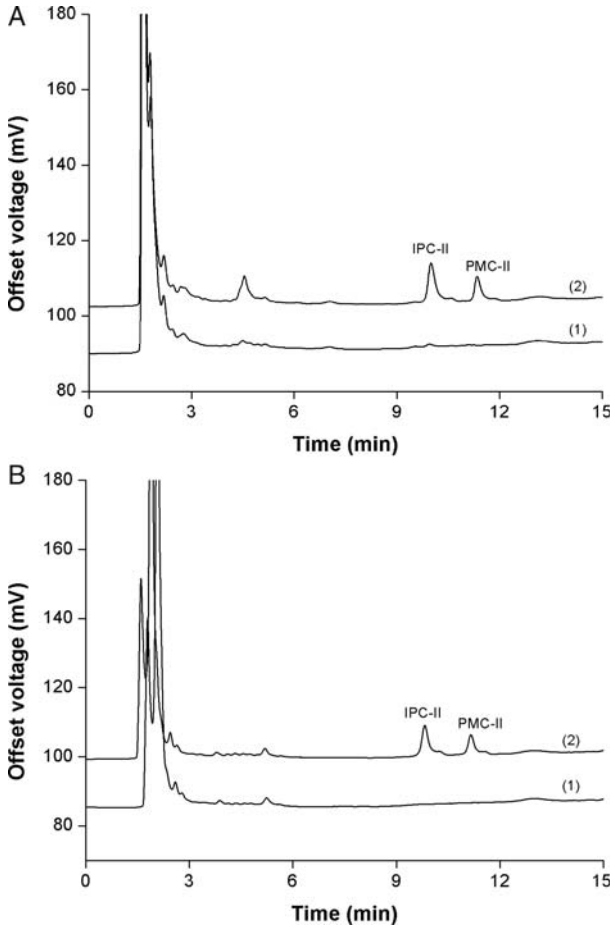


Figure 4. Typical chromatograms of (A) red grape juice 02 and (B) Bel fruit wine. Conditions: (1) blank sample, and (2) spiked 0.5 mg L^{-1} of each insecticide under the optimum conditions.

higher EF, was obtained. The correlation coefficients (R^2) of the calibrations were higher than 0.998. LODs and LOQs were evaluated based on the concentration giving a signal to noise ratio (S/N) of 3 and 10, respectively. The CPE method using Na_2CO_3 as salt additive improved the sensitivity of isoprocarb and promecarb, providing LODs of 10 and 20 μg/L, respectively, while the method without preconcentration (direct analysis) gave LODs of 0.3 and 0.5 mg/L, respectively. It is obvious that the obtained LODs were nearly 30 times lower than those obtained from our previous CPE method using NaCl as salt additive (19).

Precisions (intra-day and inter-day) of the method were examined. Intra-day precision ($n = 9$) and inter-day precision ($n = 3 \times 4$ days) were evaluated by replicate injections of 0.10 mg/L of each analyte and expressed in terms of relative standard deviation (RSD) of t_R and peak area. Good precisions with RSD $< 1\%$ and $< 8\%$ were obtained for t_R and peak area, respectively.

Extraction performance of the CPE

To evaluate the performance of the CPE − Na_2CO_3 method, a series of standard mixture solutions (0.10 and 0.50 mg/L each) was studied by replicate re-extractions under the same CPE conditions. The SRP (after the first CPE) was analyzed. Afterwards, the AQ solution from the first CPE was re-extracted (second CPE) and analyzed. The results indicated that the extraction recovery of analytes obtained after the first CPE was relatively high, in the range of 93.7–100.6% for both analytes. The concentrations and recovery obtained after the second CPE indicated that trace amounts of analytes remained in the aqueous phase. Therefore, it can be concluded that the proposed method with a single CPE step is effective enough to quantitatively extract the analytes.

Table III
Recovery of the Studied Insecticides Spiked in Fruit Juice, Vegetable Juice and Wine Samples ($n = 3$)

Sample	Concentration added (mg/L)	% Recovery (mean ± SD)	
		IPC − II	PMC − II
Fruit juice			
Red grape 01	0.050	107.0 ± 1.0	108.0 ± 1.7
	0.10	109.2 ± 5.0	107.8 ± 6.2
	0.50	110.9 ± 6.7	110.1 ± 5.0
Red grape 02	0.050	108.4 ± 8.9	93.6 ± 1.0
	0.10	108.9 ± 11.5	99.8 ± 1.3
	0.50	115.2 ± 5.4	112.8 ± 4.9
Pomegranate	0.050	107.1 ± 4.3	105.3 ± 5.7
	0.10	108.0 ± 2.0	105.0 ± 6.2
	0.50	109.3 ± 1.5	106.8 ± 3.0
Mangosteen	0.050	100.0 ± 7.9	87.6 ± 5.9
	0.10	101.3 ± 2.5	87.3 ± 1.5
	0.50	104.3 ± 6.2	102.4 ± 4.6
Vegetable juice			
Tomato	0.050	67.7 ± 3.0	71.9 ± 4.6
	0.10	99.3 ± 3.3	97.6 ± 6.9
	0.50	106.9 ± 5.4	110.6 ± 1.9
Broccoli	0.050	110.1 ± 8.8	100.3 ± 4.4
	0.10	105.4 ± 3.6	105.7 ± 9.4
	0.50	105.4 ± 4.5	115.9 ± 4.3
Mixed vegetables 01	0.050	99.7 ± 8.5	90.7 ± 8.9
	0.10	84.6 ± 1.2	76.5 ± 6.4
	0.50	105.4 ± 2.2	82.0 ± 2.6
Mixed vegetables 02	0.050	90.9 ± 2.1	—*
	0.10	87.2 ± 5.9	98.8 ± 10.1
	0.50	104.4 ± 7.1	90.6 ± 4.8
Carrot	0.050	63.9 ± 0.8	92.8 ± 9.6
	0.10	78.9 ± 3.2	83.9 ± 6.7
	0.50	78.7 ± 4.4	86.9 ± 2.3
Wine			
Bel fruit	0.050	87.4 ± 5.4	97.4 ± 6.3
	0.10	92.4 ± 5.0	87.0 ± 3.1
	0.50	110.6 ± 2.2	105.6 ± 2.0
Jambolan	0.050	89.9 ± 12.3	93.1 ± 1.5
	0.10	101.6 ± 8.3	84.8 ± 4.6
	0.50	91.3 ± 1.2	88.9 ± 3.3
Red grape	0.050	67.2 ± 2.1	65.4 ± 4.0
	0.10	84.8 ± 3.9	72.5 ± 4.0
	0.50	95.4 ± 7.1	87.2 ± 4.4
Mao berries	0.050	74.8 ± 1.9	78.9 ± 8.2
(Thai blueberries)	0.10	79.2 ± 2.6	69.5 ± 6.7
	0.50	107.4 ± 3.4	101.6 ± 2.7
Kraton	0.050	91.6 ± 7.4	70.0 ± 1.7
	0.10	99.8 ± 11.4	80.3 ± 10.3
	0.50	116.7 ± 0.4	116.3 ± 1.2

*—: not evaluated.

Application to real samples

No residues of isoprocarb and promecarb insecticides were found in the real samples (fruit juices, vegetable juices and wines). Peak identification and confirmation were carried out using retention time matching and by spiking known standards at certain concentrations. The analysis of fruit juice and

vegetable juice samples could be performed directly after filtration. In the case of wine samples, which contained an ethanol content of approximately 12% (*v*/*v*) in the original samples. The effect of alcohol on the extraction efficiency of CPE was further investigated by varying the dilution factor of the sample (Bel fruit wine was as the representative sample) corresponding to different ethanol contents. It was found that higher ethanol contents in samples decrease extraction efficiency (data not shown). The decrease of CPE efficiency can be attributed to the fact that ethanol has a high solubility in water, and thus the solution will be less polar, containing high percentages of ethanol. The adsorption of alcohol at the surface micelle–water interface imposes a restriction to the micellization of surfactant molecules, which leads to an increase in CPT (29, 30). Therefore, the analytes are less soluble in the micellar aggregates, resulting in lower recoveries. It was also found that CPE extraction can be carried out with good recoveries (80 − 100%) for the samples containing ethanol lower than 4% (*v*/*v*). However, the method can be applied to samples containing an ethanol content of up to 6% (*v*/*v*) with acceptable recoveries (70 − 100%). In our study, 1 mL of wine samples was diluted 10 times [corresponding to 1.2% (*v*/*v*) alcohol content before CPE] for analysis.

To ensure that the proposed method can be applied for the analysis of these analytes in real samples, LOD was investigated. LOD [S/N = 3] was 0.02–0.04 (IPC − II) and 0.03–0.05 mg/L (PMC − II) for fruit juice and vegetable juice samples, whereas in wine samples, LOD was 0.02–0.05 mg/L and 0.03–0.10 mg/L for IPC − II and PMC − II, respectively. The obtained LODs are acceptable with respect to the MRLs of carbamates in fruit samples (0.3–3 mg/kg) (19).

To test the reliability of the proposed methodology for the assay of isoprocarb and promecarb in fruit juice, vegetable juice and wine samples, the recovery was examined by spiking known amounts (0.05, 0.10 and 0.50 mg/L) of the standards into the samples before CPE and HPLC analysis. Typical chromatograms obtained from real samples are illustrated in Figure 4. Recoveries were satisfactory (>80%, on average) and are listed in Table III.

Conclusions

In this study, the addition of Na_2CO_3 in cloud point extraction was found to improve the extraction efficiency of isoprocarb and promecarb by (i) changing the native compounds to more hydrophobic phenolic forms (hydrolyzed forms) and (ii) promoting the salting-out effect. High extraction efficiency (>95%) was obtained for both analytes compared to those of CPE using NaCl salt additive and/or without preconcentration. Enrichment factors of up to 18 were achieved, with corresponding LODs of 10 μg/L (isoprocarb) and 20 μg/L (promecarb). The proposed method is simple, rapid and effective, and involves nontoxic organic solvents and offers good analytical features for the samples studied.

Acknowledgments

The Thailand Research Fund is gratefully acknowledged for granting the Royal Golden Jubilee (RGJ) Ph.D. Program (Grant No. PHD/0083/2549) to A. Santalad and S. Srijaranai. The authors also acknowledge the Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education for financial support.

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