Low-density solvent based ultrasound-assisted emulsification microextraction and on-column derivatization combined with gas chromatography–mass spectrometry for the determination of carbamate pesticides in environmental water samples

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A B S T R A C T

A fast and efficient method for the determination of trace level of carbamate pesticides using a lower-density-than-water solvent for ultrasound-assisted emulsification microextraction coupled to on-column derivatization and analysis by GC–MS has been developed and studied. In this approach, a soft plastic Pasteur pipette was employed as a convenient extraction device. Fifty microliters of extraction solvent, of lower density than water, was injected into the sample solution held in the pipette. The latter was immediately immersed in an ultrasound water bath to form an emulsion. After 2 min extraction, the emulsion was fractionated into two layers by centrifugation. The upper layer (organic extract) could be collected conveniently by squeezing the bulb of the pipette, now held upside down, to move it into the narrow stem of the device, facilitating its retrieval for analysis. The extract was then combined with trimethylphenylammonium hydroxide and directly injected into a gas chromatography–mass spectrometry (GC–MS) system for on-column derivatization and analysis. The on-column derivatization provided an added convenience (since a separate step was not necessary). Parameters affecting the derivatization and extraction were investigated. Under the most favorable conditions, the method demonstrated high extraction efficiency with low limits of detection of between 0.01 and 0.1 μg/L, good linearity in the range of 0.05–50 μg/L, to 0.5–100 μg/L, and good repeatability (RSD below 9.2%, $n = 5$). The proposed method was evaluated by determining carbamate pesticides in river water samples.

1. Introduction

Carbamate pesticides, esters derived from carbamic acid, are one of the major classes of highly effective commercial pesticides which are used instead of organophosphorous and organochlorine pesticides [1]. Due to their low bioaccumulation potential, effectiveness, and broad biological activity, carbamate pesticides are used worldwide in agriculture, home, and gardens for the control of insects, fungi, and weeds [2,3].

However, since they are inhibitors of anticholinesterase [4,5], carbamate pesticides are considered hazardous to human health, and they have been listed by the United States Environmental Protection Agency. Since carbamate pesticides are highly soluble in water, they pose a risk to the aquatic environment. Therefore, the monitoring and determination of trace levels of these pesticides

in environmental matrices, has received a great deal of attention [1–4].

The most commonly used analytical techniques for determining carbamate pesticides include micellar electrokinetic chromatography [6,7], capillary zone electrophoresis-with ultraviolet detection (UV) [8], high performance liquid chromatography (HPLC) coupled to UV [9–12], diode array detector (DAD) [13,14], fluorescence detection (FLD) [15], chemiluminescence detection (CL) [16], or mass spectrometry (MS) [17,18], and gas chromatography (GC)–MS [2,19–21]. With good sensitivity to obtain low detection limits and high selectivity to reduce potential interferences, as well as fast analysis, GC–MS represents a powerful detection method for carbamate pesticides.

Since they are thermally labile, before analysis by GC, carbamate pesticides need to be derivatized to form more thermally stable derivatives to avoid their breakdown to amines and phenols in the injection port [2,10,22,23]. The commonly used derivatization reactions are silylation, acetylation, and alkylation [2]. On-column derivatization is a very convenient derivatization procedure since this method is simple and involves a fast one-step operation [2].

Due to the trace-level concentration of carbamate pesticides in environmental matrices, preconcentration, prior to chromatographic analysis, is usually necessary to achieve low detection limits. Different sample preparation methods such as solid-phase extraction (SPE) [21,24,25], micro-solid-phase extraction [10], liquid–liquid extraction (LLE) [26,27], supercritical fluid extraction [9,28,29], microwave-assisted extraction [9,30], liquid-phase microextraction (LPME) [2,31,32] and solid-phase microextraction (SPME) [33–37] have been employed for the extraction of carbamate pesticides from different sample matrices. However, for most of the above mentioned methods, considerable time is required for analytes to be extracted into extraction solvents or onto sorbents. Efforts have been made on developing fast and efficient extraction methods for the preconcentration of carbamate pesticides from aquatic environment samples.

Dispersive liquid–liquid microextraction (DLLME) was developed in 2006 [38]. DLLME is fast and provides high enrichment, and has been widely used for the extraction of different water contaminants [12,14,15,38]. It typically uses tens of microliters of organic solvent as extraction solvent and another 100–200 μL of another organic solvent to facilitate the dispersion of the former in the aqueous sample.

Very recently, Garcia-Jares and his co-workers developed ultrasound-assisted emulsification microextraction (USAEME) [39]. In this method, a water-immiscible extraction solvent is dispersed into aqueous sample solution under the assistance of ultrasound to form an emulsion, without using any dispersive solvent. Compared to DLLME, the main advantage of USAEME is the avoidance of using a relatively large amount (typically, hundreds of microliters) of dispersive solvent, as well as having the usually high enrichment and speed. Since its introduction, USAEME has been applied to the extraction of a variety of organic compounds in different matrices [40–45].

However, the extraction solvents used in DLLME and USAEME are limited to those that are of higher density than water in order for them to be sedimented by centrifugation and conveniently collected after extraction. These are typically chlorinated solvents, which are undesirable from health and environmental points of view.

To overcome this limitation, several studies have reported the use of low density solvents in DLLME [46–52], to broaden the applicability of the procedure. For example, Saleh et al. [46] employed a home-designed and fabricated extraction vial that allowed the use of toluene as extraction solvent. A similar device was also employed by Farajzadeh et al. [47] that allowed the use of cyclohexane as extraction solvent. However, since such extraction devices were home-designed and fabricated, their accessibility and availability is limited. In Shi and Lee's work [48], after DLLME, the extract was adsorbed by magnetic nanoparticles; the need for these particles possibly added complexity to the overall method. In other studies [49–51], a volumetric flask or a round-bottom glass vial was used as the extraction device. However, the collection of a small volume of the extract floating on top of the aqueous sample was inconvenient since the diameter of the vessels were relatively wide, making the extract, which formed a thin layer only, difficult to retrieve. More recently [52], we reported a very simple and convenient application of low-density solvent as extraction solvent in DLLME by using a soft polyethylene Pasteur pipette, which is widely commercially available, as the extraction device.

In this work, for the first time, we employed the plastic Pasteur pipette approach for low-density solvent based USAEME (LDS-USAEME) combined with on-column derivatization, followed by GC–MS determination of trace-level carbamate pesticides. The use of a simple, widely available device that can be further developed to expand the future applicability and utility of dispersive liquid–liquid microextraction (DLLME)-based in USAEME,

combined with on-column derivatization, is advantages. The adoption of the plastic pipette in this work permitted the use of toluene, a solvent with lower density than water, as extraction solvent for the USAEME procedure. The on-column derivatization, in which the derivatization reagent was injected into GC–MS together with the extract, avoided an additional separate derivatization step and expedited the extraction and analytical procedure. Different parameters that affect the derivatization and extraction efficiency were evaluated. Under the most favorable conditions, the proposed method was applied to analyze carbamate pesticides in genuine river water samples.

2. Experimental

2.1. Chemicals and materials

The analytes, carbaryl (purity 98%), chlorpropham (purity 99.5%), methiocarb (purity 99%), carbofuran (purity 98%), promecarb (purity 99%), and propham (purity 99.5), were supplied by ChemService (West Chester, PA, USA). The structures of these analytes are listed in Table 1.

HPLC-grade methanol, acetone, chloroform, and *n*-hexane were purchased from Tedia Company (Fairfield, OH, USA). Toluene and cyclohexane were from Fisher (Loughborough, UK) while 1-octanol was bought from Merck (Darmstadt, Germany). Trimethylphenylammonium hydroxide (TMPAH) was purchased from Supelco (Bellefonte, PA, USA). The *o*-xylene was obtained from Sigma–Aldrich (St. Louis, MO, USA). Sodium chloride (NaCl) was acquired from Goodrich Chemical Enterprise (Singapore). Ultrapure water was produced on a Nanopure water purification system (Barnstead, Dubuque, IA, USA).

The centrifuge (model 5810R) was from Eppendorf (Hamburg, Germany). The soft polyethylene Pasteur pipette (5-mL capacity) was manufactured by Continental Lab Products (San Diego, CA, USA) and was purchased from Practical Mediscience Pte., Ltd (Singapore). An ultrasonic water bath was from Soniclean PTY. Ltd (Thebarton, S.A., Australia). A 100 μL syringe used for injection of extraction solvent, and a 50 μL blunt tip microsyringe used for collection of the organic extract were purchased from Hamilton Bonaduz AG (Bonaduz, Switzerland). A 10 μL microsyringe used for GC–MS injection was bought from SGE (Sydney, Australia).

2.2. GC–MS analysis

Sample analyses were carried out on a Shimadzu (Kyoto, Japan) QP2010 GC–MS system equipped with a Shimadzu AOC-20i autosampler and a DB-5 MS (J&W Scientific, Folsom, CA, USA) fused silica capillary column (30 m × 0.25 mm internal diameter (i.d.), 0.25 μm film thickness). Helium (purity 99.9999%) was employed as the carrier gas at a flow rate of 1.7 mL/min. Samples (1 μL) were injected in splitless mode. The injector temperature was set at 280 °C and the interface temperature maintained at 300 °C. The GC oven was initially held at 60 °C for 2 min and then programmed to 260 at 10 °C/min; held for 2 min. The solvent cut time was 8.5 min. Carbamate pesticide standards and samples were analyzed in selective ion monitoring (SIM) mode for quantitative determination of the analytes. The masses monitored by the detector were set as follows: promecarb, *m/z* 164, 149; carbofuran, *m/z* 178, 163; propham, *m/z* 193, 151, 106; carbaryl, *m/z* 158, 115, 143; methiocarb, *m/z* 182, 167, 152; chlorpropham, *m/z* 227, 185, 141. The monitored ions (in SIM mode) of the derivatives were selected based on good selectivity and high sensitivity. The mass spectra of derivatives of these carbamate pesticides are shown in Fig. 1.
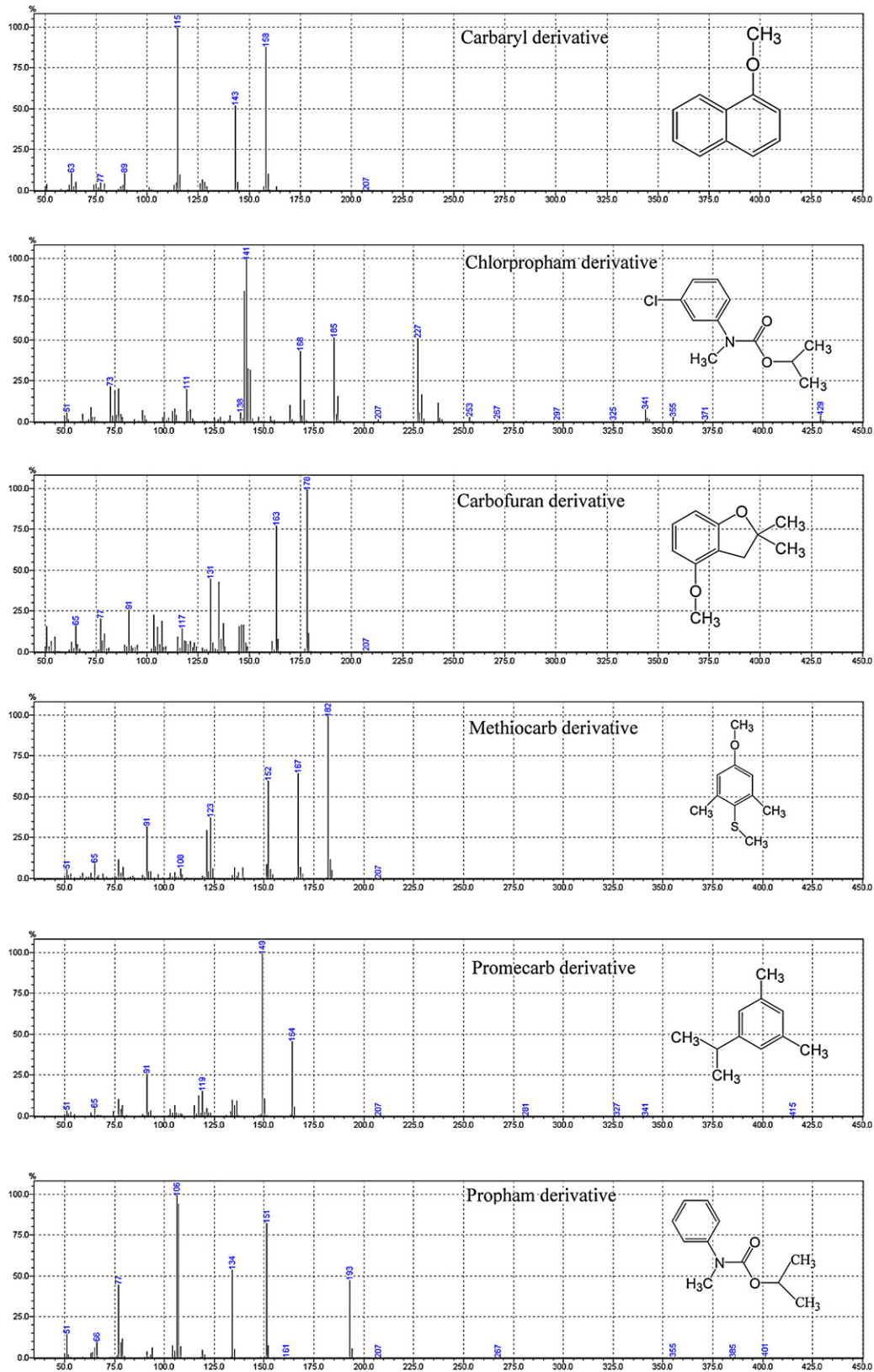
Fig. 1. Mass spectra of carbamate pesticide derivatives.

2.3. Sample preparation

A stock solution (containing 1000 mg/L of each analyte) was prepared in methanol and diluted with methanol at different concentrations to obtain standard solutions, from which calibration plots were prepared. All solutions were stored in the refrigerator at 4 °C. Water samples were prepared by spiking ultrapure water with the analytes at known concentrations.

Table 1
Chemical structures of carbamate pesticides considered in this work.

Analyte	CAS number	Structure
Promecarb	2361-65-7	
Carbofuran	1563-66-2	
Propham	122-42-9	
Carbaryl	63-25-2	
Methiocarb	2032-65-7	
Chlorpropham	101-21-3	

Genuine river water samples were collected from a local river into pre-cleaned glass bottles and transported to the laboratory immediately. All collected water samples were kept in the dark at 4 °C before use. The samples were extracted and analyzed without any prior treatment or filtration to avoid possible loss of the target analytes.

2.4. LDS-USAEME with on-column derivatization

Fig. 2 shows the LDS-USAEME procedure. In the extraction procedure, a 5-mL soft polyethylene Pasteur pipette was filled with 4 mL of water sample. Fifty microliters of the extraction solvent were injected into the sample solution. The pipette was then immersed in an ultrasonic water bath. The extraction was
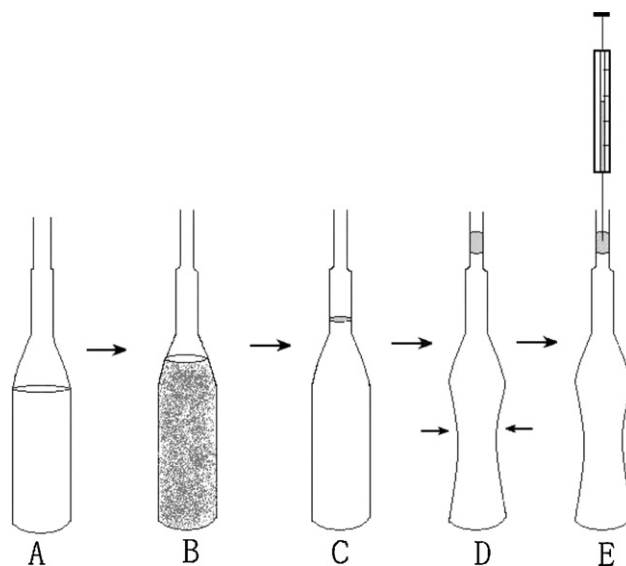

Fig. 2. The LDS-USAEME procedure.

performed at 25 °C (ambient temperature). Under the ultrasonication, the extraction solvent was dispersed into the sample solution to form an emulsion, in which analyte extraction took place into the highly dispersed micro droplets of the extraction solvent. After 2 min of extraction, the emulsion was separated into two phases by centrifugation at 4000 rpm for 4 min. The pipette was then held upside down and its bulb squeezed slightly and gently to move the upper layer (low density organic extract) into its narrow stem. This enabled the convenient collection of the extract using a 50 μL GC microsyringe. One microliter of the extract combined with 1 μL of derivatization reagent was then injected into the GC–MS system for analysis.

2.5. Conventional USAEME

Four microliters of aqueous sample was placed in a 10-mL conical centrifuge tube, and then a 50 μL of CHCl₃ was added as extraction solvent. The mixture was immersed into an ultrasound water bath for extraction at 25 °C. After 2 min of extraction, the emulsion was broken up by centrifugation at 4000 rpm for 4 min. The organic extract sedimented at the bottom of the centrifuge tube was collected and then 1 μL combined with 1 μL of derivatization reagent was injected into GC–MS for analysis. The conditions used were based on our own experience as well as results from previous publications [3,22].

2.6. LDS-DLLME

To carry out LDS-DLLME, 4 mL of water sample was placed in a polyethylene Pasteur pipette. A mixture of 500 μL acetone (serving as dispersive solvent) and 50 μL toluene (serving as extraction solvent) was rapidly injected into the aqueous solution. After 2 min of extraction, the emulsion was centrifuged at 4000 rpm for 4 min to afford two separate phases. The upper layer (organic extract) was collected as described above, and 1 μL of the extract with 1 μL of derivatization reagent were injected into the GC–MS system for analysis. The conditions used were based on our own experience as well as results from previous work [49].
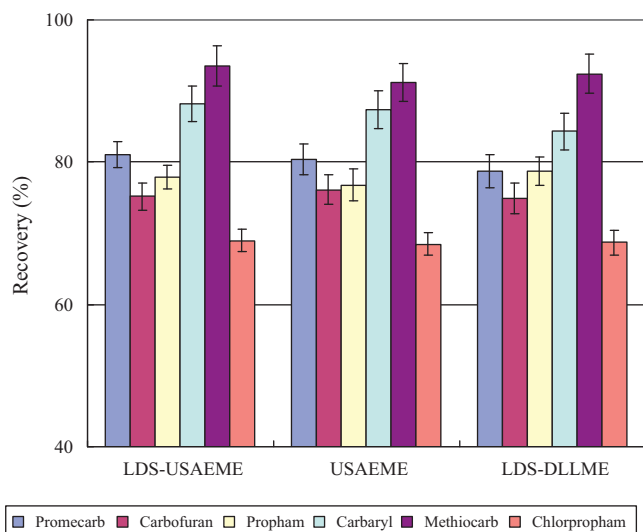
Fig. 3. Comparison of LDS-DLLME, USAEME, and LDS-USAEME.

Fig. 4. Effect of derivatization reagent volume on extraction.

3. Results and discussion

3.1. Comparative studies

To compare the performance of LDS-USAEME, conventional USAEME and low-density solvent based DLLME (LDS-DLLME) were selected as references. Spiked ultrapure water samples (5 μg/L of each analyte) were used.

Fig. 3 shows that the extraction efficiencies obtained by all three approaches were comparable. However, LDS-USAEME has some conceivable advantages. Most importantly, no dispersive solvent was needed in LDS-USAEME. In DLLME, this usually amounts to hundreds of microliters. As is well known, this is the most prominent feature of USAEME compared to DLLME. Furthermore, the toluene employed in LDS-USAEME in the present work, is generally much less toxic than chlorinated solvents widely used in conventional USAEME. The proposed method offers a simple and practical approach to extend the range of suitable solvents for USAEME use, overcoming the limitation of high density chlorinated extraction solvents necessary for conventional DLLME with centrifugation in order to set down the final extract at the bottom of the extraction vessel.

3.2. Derivatization

Depending on their structures, carbamate pesticides react with TMPAH in two different ways [2,53]: (1) N-arylcarbamates, including propham and chlorpropham, yield N-methyl-N-arylcarbamate products,

$$Ar-NH-CO-O-R \rightarrow Ar-N(CH_3)-CO-O-R$$
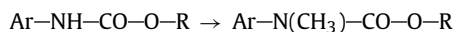
and (2) N-methylcarbamates, including promecarb, carbaryl, methiocarb, and carbofuran, yield methyl substituted products (arylmethyl ethers),

$$Ar-O-CO-NH-CH_3 \rightarrow Ar-O-CH_3$$
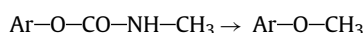
where Ar is aryl. The mass spectra of the carbamate pesticide derivatives are shown in Fig. 1.

The volume of TMPAH is important for the derivatization. A series of experiments was carried out to study the effect of different volumes of TMPAH on derivatization. Different volumes (1, 2, 5, 10, 20, and 40 μL) of TMPAH were applied to derivatize 10 μL of a standard solution (of an individual carbamate at 1 mg/L
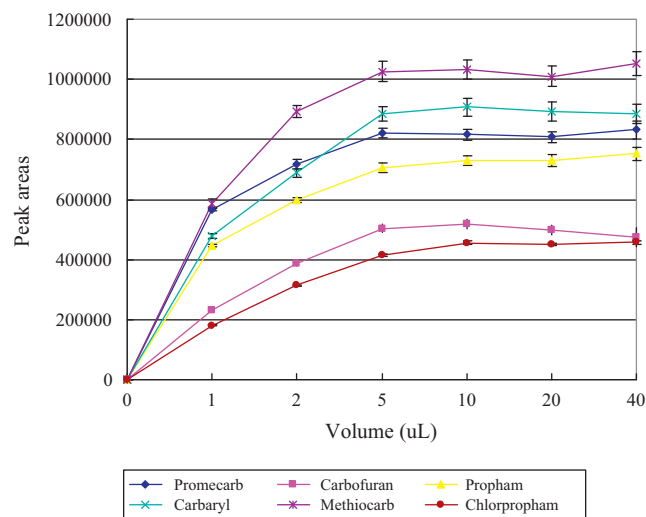
concentration). The results are shown in Fig. 4. It may be observed that the peak areas for all derivatives increased rapidly with the increase of volume of TMPAH, up to ca. 5 μL. Above 5 μL, the peak areas for all derivatives flattened out. Considering that the higher volume of derivatization reagent had no negative effect on the derivatization, 10 μL of derivatization reagent was selected to ensure the reagent was in excess for successful derivatization. Therefore, the volume ratio of 1:1 for organic extract and derivatization reagent was adopted for subsequent experiments.

3.3. Optimization

In USAEME, the extraction efficiency depends greatly on the extraction solvent and its volume, as well as other parameters including ultrasonic time, temperature, the ionic strength of the sample solution, and the speed and time of centrifugation. All these parameters were investigated to determine the most favorable conditions of the developed method. The extraction recovery was considered to evaluate the influence of the different parameters on extraction efficiency. All experiments were performed in triplicate.

The extraction recovery, R, was calculated using the following equation:

$$R = \frac{C_O V_O}{C_0 V_{aq}} \times 100\%$$

where C_O, C_0, V_O, and V_{aq} are the concentration of analytes in the upper layer (extract), the spiked concentration of analytes in the aqueous solution, the volume of the upper layer, and the volume of the aqueous solution, respectively.

3.3.1. Extraction solvent

There are some conditions that need to be met in selecting an extraction solvent. First of all, the extraction solvent should have high extraction capability for the target analytes. Secondly, the extraction solvent should have low solubility in aqueous solution. Thirdly, for this proposed method, the extraction solvent should have lower density than water such that it could be directly withdrawn as the upper layer from the Pasteur pipette stem after centrifugation. Five low density solvents [50] that satisfy these requirements, n-hexane (density, 20 °C, d = 0.66 g/mL), cyclohexane (d = 0.78 g/mL), o-xylene (d = 0.88 g/mL), toluene (d = 0.87 g/mL) and 1-octanol (d = 0.82 g/mL), were used here to evaluate their performance.
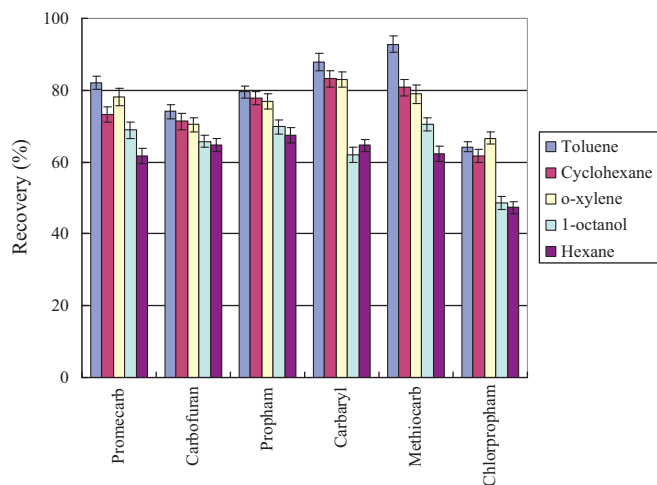
Fig. 5. Effect of type of extraction solvent on extraction.

Fig. 6. Effect of temperature on extraction.

Based on their different solubilities in water, different initial volumes of extraction solvents were used in order to achieve an equal volume in the upper layer after centrifugation. As it can be seen in Fig. 5, toluene has the highest extraction efficiency for most of the compounds (except for chlorpropham), followed by cyclohexane and o-xylene, and then, 1-octanol and n-hexane. This may be partly due to the aromatic group and/or ring structures in the solvents which benefit the extraction ("like dissolves like") [49,50]. Additionally, the low viscosity of toluene contributes to the efficient formation of the emulsion, thus facilitating the extraction.

3.3.2. Volume of the extraction solvent

During USAEME, the volume of the extraction solvent is a key parameter. This effect was investigated by performing the extraction using a series of toluene volumes (30, 40, 50, 60, and 70 µL). The enrichment factors decreased with increasing the volume of toluene from 30 to 70 µL. This is expected due to the dilution effect of the analytes at a higher volume of extraction solvent. On the other hand, it was very difficult to collect the upper layer when the initial volume of extraction solvent was less than 30 µL. With the primary focus on high enrichment factor, a volume of no higher than 50 µL was used for subsequent experiments.

3.3.3. Extraction temperature

In LPME, in general, the temperature usually has a significant effect on the extraction efficiency. Generally speaking, increase of the extraction temperature can lower the distribution coefficient and increase the diffusion coefficient, thus facilitating mass transfer of the analytes from the aqueous solution to the organic solvent.

In order to examine the effect of temperature, a series of experiments was performed at 25 °C (ambient temperature), 30, 40, 50, and 60 °C, respectively. The results (shown in Fig. 6) demonstrated that the extraction efficiency was independent of temperature. This may be explained from two aspects: high temperature caused faster mass transfer of analytes, resulting in the increase of extraction. On the other hand, a high temperature could favor the partial dissolution of the extraction solvent in the aqueous sample solution, reducing the extraction efficiencies, as reported previously [54,55]. It is likely that the final outcome depends on the predominance of one factor over the other (analogous to solid-phase microextraction (SPME) where higher extraction temperature can lead to opposing effects (increased partition coefficients (higher extraction) versus greater desorption (lower extraction), since SPME is an exothermic process). In the present case, it appears that a balance was applicable, and temperature was not observed to have an
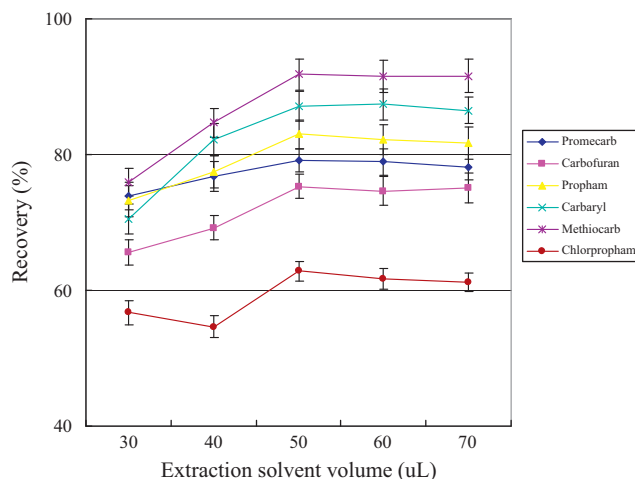
effect on extraction efficiency. For practicality, simplicity and convenience, therefore, extraction was conducted at 25 °C (ambient temperature).

3.3.4. Extraction time profiles

Usually, in LPME and SPME, long extraction time favors the attainment of extraction equilibrium, and may lead to better extraction performance. In USAEME, the extraction time is defined as the interval between the beginning of the emulsification and the time at which the emulsion is centrifuged [46]. The effect of time (1, 2, 5, 10, and 20 min) on the extraction efficiency was investigated. Fig. 7 shows the extraction time profiles. It can be seen that, the extraction time has no significant effect on extraction efficiency. The extraction reached a maximum in less than 1 min, and then, remained almost constant as extraction time was prolonged. Considering the need to exploit the speed of the extraction process, the extraction time was set at 2 min.

3.3.5. Effect of ionic strength

The effect of ionic strength on the extraction efficiency was evaluated by increasing the concentration of sodium chloride in the sample solution. There was no observably change on the extraction efficiency for all analytes with the variation of sodium chloride concentration from 0 to 30% (w/v). This result was in accordance with that obtained in a previous study [22]. This observation may be the result of some competitive effects. According to the salting-out
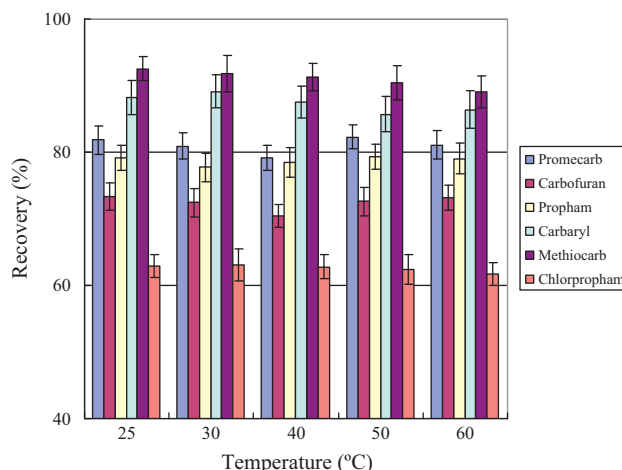

Fig. 7. Extraction time profiles.

Table 2
Linear range, limits of detection, limits of quantification, recovery, and precision of LDS-USAEME combined with on-column derivatization and GC–MS analysis of carbamate pesticides.

Analyte	Linear range (μg/L)	Correlation coefficient (r)	LODs (μg/L)	LOQs (μg/L)	Recovery (%)	RSD (%, n = 5)
Promecarb	0.1–50	0.9931	0.02	0.06	82.7	5.3
Carbofuran	0.5–100	0.9909	0.1	0.3	74.5	8.6
Propham	0.2–100	0.9926	0.05	0.15	78.2	6.9
Carbaryl	0.1–50	0.9953	0.02	0.06	88.0	7.8
Methiocarb	0.05–50	0.9982	0.01	0.03	93.1	3.7
Chlorpropham	0.5–50	0.9917	0.1	0.3	68.9	9.2

effect, the partition of analytes from the aqueous sample into the extraction solvent will increase with the addition of more sodium chloride. However, with the increased amount of sodium chloride, the viscosity and density of the solution were enhanced, decreasing the mass transfer of analytes, thus diminishing the extraction efficiency. In addition, in a viscous solution, the ultrasound waves can be absorbed and dispersed as calorific energy, drastically reducing the emulsification phenomenon [39,56]. Thus, no salt was added in subsequent experiments.

3.3.6. Time and speed of centrifugation

In USAEME, centrifugation was essential to break up the emulsion and separate the extract from the aqueous solution. The time and speed of centrifugation would affect the extraction efficiency.

A series of centrifugation parameters were studied in terms of the time and speed to obtain complete fractionation of the upper layer. Firstly, different centrifugation times (1–15 min) were evaluated while centrifugation speed was maintained at 4000 rpm. The results (not shown) indicated that, as the centrifugation time increased from 0 to 5 min, the extraction efficiency increased. After 5 min, the extraction efficiency flattened out until ca. 10 min, after which the extraction decreased slightly gradually, conceivably due to the evaporation of the upper layer [46]. Therefore, 5 min was adopted as centrifugation time. Secondly, different centrifugation speeds were investigated. As expected, higher centrifugation speeds facilitated the complete separation of emulsion in a shorter time. However, to avoid possible deformation of, or damage to the polyethylene pipette under higher-speed centrifugation, a maximum value of 4000 rpm was used.

Based on the above discussion, the most favorable extraction and derivatization conditions for USAEME were as follows: 50 μL of toluene as extraction solvent, ultrasonication for 2 min at 25 °C, centrifugation at 4000 rpm for 5 min, no salt addition. Injection volume for GC–MS analysis of 1 μL of extract combined with 1 μL of

TMPAH. All the following experiments were performed under these conditions.

3.4. Method validation

Under the described extraction conditions, the linearity range, limits of detection (LODs), limits of quantification (LOQs), repeatability, and recoveries were measured using spiked ultrapure water samples. Results of the validation parameters are shown in Table 2.

The linearity of the method was studied with a series of concentrations, and was obtained in the range of 0.05–50 μg/L for methiocarb, 0.1–50 μg/L for promecarb and carbaryl, 0.2–50 μg/L for propham, and 0.5–50 μg/L for carbofuran and chlorpropham. Correlation coefficients higher than 0.9909 were obtained for all analytes. The repeatability of the method was studied by five replicate analyses of the spiked samples under the same operational parameters, and presented as the relative standard deviations (RSDs), which were between 3.7% and 9.2%, showing the good repeatability of the method.

The LODs, defined at a signal-to-noise (S/N) ratio of 3, were obtained in the range of 0.01–0.1 μg/L. The LOQs, defined at S/N = 10, were from 0.03 μg/L to 0.3 μg/L.

From Table 3, it can be seen that the LODs achieved by this method were lower than those obtained with LPME on-column derivatization-GC–MS [2], DLLME-HPLC–DAD [3], automated SPE-LC–CL [16], surfactant-enhanced USAEME-HPLC–DAD [22], dispersive SPE-LC–MS [18], and comparable with the values attained by SPE-SPME-GC–MS [23], SPE-LLME-HPLC–DAD [13], and direct immersion-SPME-GC–MS [57], and higher than those with solvent terminated DLLME-GC–MS [49], SPE-LC–MS [58], and pressurized liquid extraction-GC–MS [19]. It is noted that the last three mentioned techniques exhibiting lower LODs than LDS-USAEME required relatively larger amount (from 1 to 10 mL) of organic solvents for extraction (Table 3).

The LODs indicated that the proposed method have satisfactory sensitivity and could be fully applied to the determination of

Table 3
Comparison of LODs with different methods.

Method	Analyte	Solvent and volume	LODs (μg/L)	Ref.
LPME on-column derivatization-GC–MS	Carbamate pesticides	1-Octanol, 2 μL	0.2–0.8	[2]
DLLME-HPLC–DAD	Carbamate pesticides	Chloroforum 70 μL and acetone 930 μL	1.3–3.3	[3]
SPE-LC–CL	N-methyl carbamate pesticides	Acetonitrile 2 mL	4–42	[16]
Surfactant-enhanced USAEME-HPLC–DAD	Carbamate pesticides	Chlorobenzene–chloroform 150 μL and tween20 30 μL	0.1–0.3	[22]
Dispersive SPE-LC–MS	Carbamate and organophosphorous pesticides	Acetonitrile 10 mL	0.5–35 μg kg^{-1}	[18]
SPE-SPME-GC–MS	Carbamate pesticides	Acetonitrile 2 mL	0.05–0.46	[23]
SPE-LLME HPLC–DAD	Pesticides (acidic, basic, and neutral)	Acetonitrile 1.5 mL and dichloromethane 7.5 mL	0.04–4.43	[13]
Direct immersion SPME-GC–MS	Carbamate pesticides etc.		0.02–0.07	[57]
Solvent terminated DLLME-GC–MS	Carbamate pesticides	Toluene 15 μL and acetonitrile 1.0 mL	0.001–0.05	[49]
SPE-LC–MS	Carbamate pesticides etc.	Dichloromethane–methanol (1:1) 10 mL	0.0005–0.0055	[58]
Pressurized liquid extraction-GC–MS	Carbamate pesticides etc.	n-Hexane–ethyl acetate (80:20) 7.7 mL	0.3–3.0 pgg^{-1}	[19]
LDS-USAEME	Carbamate pesticides	Toluene 50 μL	0.01–0.1	This work

Table 4
Summary of results of LDS-USAEME combined with on-column derivatization and GC–MS analysis of carbamate pesticides in spiked genuine river water sample.

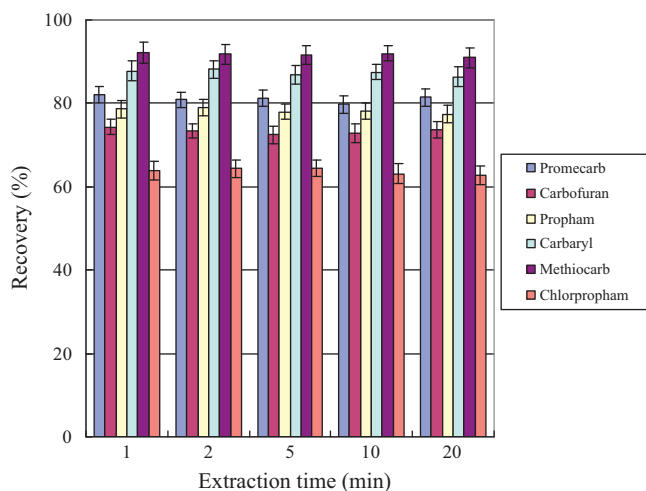| Analyte	Spiked river water (2 µg/L of each analyte)		Spiked river water (20 µg/L of each analyte)	
	Relative recovery (%)	RSD (%)	Relative recovery (%)	RSD (%)
Promecarb	91.4	7.5	87.9	6.9
Carbofuran	88.9	8.8	95.4	9.0
Propham	107.1	7.4	102.1	6.8
Carbaryl	93.6	6.1	98.6	5.7
Methiocarb	98.3	4.7	100.7	5.5
Chlorpropham	103.5	8.5	108.3	9.7

Fig. 8. Chromatogram of spiked river water sample extracted by LDS-USAEME under the most favorable conditions as described in the text. (1) Promecarb, (2) Carbofuran, (3) Propham, (4) Carbaryl, (5) Methiocarb, and (6) Chlorpropham.

carbamate pesticides at trace level concentrations in water samples.

3.5. Genuine water sample analysis

To evaluate the applicability of the current method, it was applied to determine carbamate pesticides (spiked 2.0 and 20 µg/L of each analyte) in genuine river water samples since carbamates are not officially used in this country, and the samples are not likely to contain the contaminants. The river water samples were extracted and the analytes derivatized on-column using the developed method before analysis by GC–MS. The relative recoveries, defined as the ratios of the peak areas of the analytes in real water samples and peak areas of analytes in ultrapure water samples spiked with the same amount of the analytes, and which serve to indicate matrix effects, were calculated. The results are listed in Table 4. It can be observed that the relative recoveries were between 87.9% and 108.3% for all analytes, suggesting that the genuine river water matrices have minor effects on the developed method. The results demonstrated that the method described in the present work is suitable for the fast analysis of trace levels of carbamate pesticides in genuine water samples. Fig. 8 shows a chromatogram of spiked genuine river water sample (2.0 µg/L of each analyte) extract under the most favorable extraction and derivatization conditions as described above.

4. Conclusion

This work demonstrated a fast, simple, and efficient method to extract and analyze carbamate pesticides at trace levels in river water samples. Employing a disposable plastic Pasteur pipette as extraction device, a lower-density-than-water solvent was utilized as the extraction solvent in ultrasound-assisted emulsification microextraction (LDS-USAEME). This approach affords operational convenience and practicality in using lower-density-than-water solvents in USAEME, making it easier to expand the range of available solvents for this DLLME method since higher-density-than-water solvents, which are generally highly undesirable to operators or the environment, are usually used in USAEME. The use of the pipette enabled the expedient retrieval of the extract. It should also be emphasized that by applying the use of this type of pipette that is easily available for purchase from numerous suppliers, the need for any other fabricated device for DLLME using less-dense-than-water solvents, amongst other DLLME-related methods, is completely eliminated. This makes the analytical procedure completely accessible to any laboratory, everywhere. Furthermore, LDS-USAEME was rapid (less than 10 min), as was the GC–MS analysis (~13 min). Also, the combination of LDS-USAEME with on-column derivatization simplified the operation significantly. The method offers good LODs in the sub-parts-per-billion level, as well as good linearity and acceptable repeatability. The LDS-USAEME with on-column derivatization and GC–MS analysis was shown to be a fast, efficient, and practical approach for the determination of carbamate pesticides in aqueous samples. That LDS-USAEME based on the soft Pasteur pipette can be considered for onsite operations should also be acknowledged.

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