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Condensation nucleation light scattering detection with ion chromatography for direct determination of glyphosate and its metabolite in water

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Abstract

An ion chromatography–condensation nucleation light scattering detection (IC–CNLSD) method was successfully used to directly analyze glyphosate, a polar pesticide, and aminomethylphosaphonic acid, the major metabolite of glyphosate, in water without need of pre-treatment or derivatization. CNLSD gave a LOD of 53 ng/ml for glyphosate, which is much lower than the maximum contaminant level of 700 ng/ml for drinking water issued by the US Environmental Protection Agency. Spiked analytes in different matrixes were tested. A diluted commercial herbicide containing glyphosate was also evaluated. Compared to other reported methods, the IC–CNLSD method has no need of sample derivatization, pre-concentration, and mobile phase conductivity suppression. It is simple, fast and inexpensive. IC–CNLSD is an ideal direct detection technique for such pesticides without chromophores or fluorophores.

Keywords: Condensation nucleation light scattering detection; Detection, LC; Glyphosate; Pesticides; Aminomethylphosaphonic acid

1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine, Fig. 1] is a broad-spectrum, non-selective, post-emergence herbicide introduced by Monsanto in the early 1970s. The physical, chemical and toxicological properties of glyphosate have been well reviewed [1]. Because of its low mammalian toxicity, glyphosate is widely used for vegetation control, and it is in the top rank among conventional pesticides used in the USA. The maximum contaminant level (MCL) of glyphosate for safe drinking water issued by the US Environmental Protection Agency (EPA) is 0.7 μg/ml [2]. Therefore, to establish a fast, simple and sensitive assay method for glyphosate and AMPA (aminomethylphosaphonic acid, Fig. 1), the only major metabolite of glyphosate in plant, water and soil, in the environment would be beneficial.

Belonging to the amino acid class of pesticides, glyphosate is amphoteric, very polar, highly water-soluble and non-volatile. At the same time, its lack of a chromophore or fluorophore produces considerable challenge to traditional analytical techniques to

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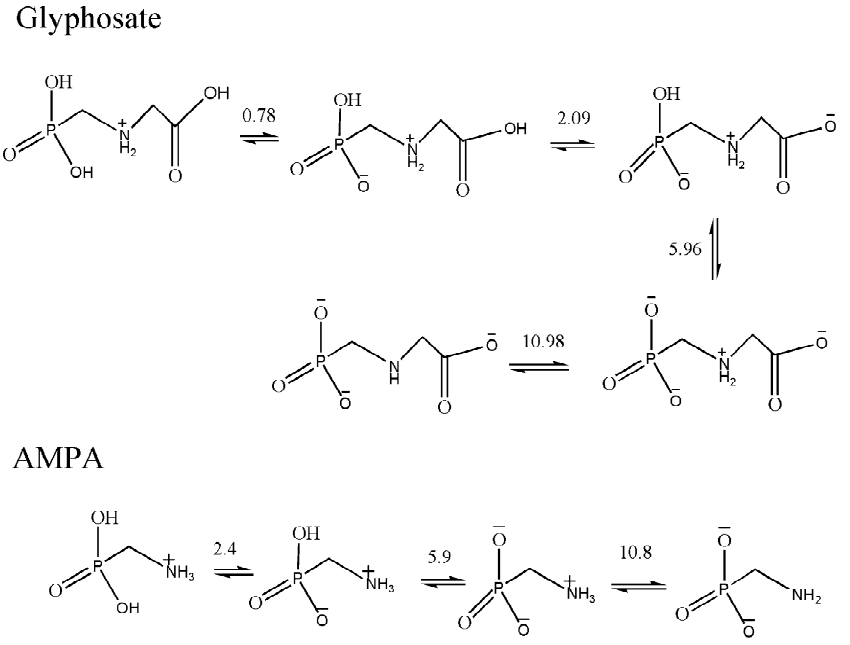

Fig. 1. Structures and ionization processes of glyphosate and AMPA.

develop a simple and sufficiently sensitive method for the determination of this compound.

Analytical methods to determine phosphonic and amino acid group-containing pesticides have been reviewed recently [3]. Gas chromatography (GC) analysis [4] of glyphosate and AMPA requires an exhaustive derivatization step to convert each substance into a less polar and more volatile derivative before separation.

High-performance liquid chromatography (HPLC) is more practical than GC for the separation of glyphosate and AMPA because it is more suitable for aqueous and non-volatile samples. Detection is the essential problem here. The absence of chromophores or fluorophores makes it impossible to detect these substances with reasonable sensitivity by HPLC without derivatization. Both pre-column and post-column derivatization techniques have been investigated. Pre-column procedures generally use 9-fluorenylmethyl chloroformate (FMOC-Cl) as the derivatization reagent to form a glyphosate–FMOC derivative, which is amenable to fluorescence detection [5,6]. Other derivatization reagents, such as 1-fluoro-2,4-dinitrobenzene [7] and *p*-toluene sulfonylchloride [8] have also been studied. The reported post-column derivatization reagents included *o*-phthalaldehyde–2-mercaptoethanol [9], ninhydrin [10] and Al^{3+}-morin (3,5,7,2′,4′-pentahydroxylflavone) [11].

Very few detection methods for glyphosate and AMPA without derivatization have been reported. Zhu et al. [12] developed a suppressed conductivity ion chromatography (IC) method for analyzing glyphosate with the limit of detection (LOD) of 42 ng/ml. Bauer et al. [13] integrated a suppressor module into an IC–MS–MS system to analyze polar organic compounds in water, including glyphosate and AMPA. A new enzyme-linked immunosorbent assay approach [14] was developed to analyze glyphosate in water. This method is faster and simpler than GC and HPLC methods, but with an LOD of 7.6 μg/ml, the method sensitivity is not good enough to be applied practically without sample preconcentration.

Condensation nucleation light scattering detection (CNLSD) is a recently developed detection technique [15,16] which has been coupled with different separation techniques including HPLC [17–20], supercritical fluid chromatography [21], capillary electrophoresis [22,23] and capillary electrochromatography [24]. Because of its universality, sensitivity, low cost and simple operation when combined with

suitable separation methods, CNLSD would be expected to be a useful detection system for polar pesticides without chromophores and fluorophores, without further derivatization and preparation steps.

2. Experimental

2.1. Chemicals

Glyphosate and AMPA were purchased from Sigma (St. Louis, MO, USA). Ultrex ultrapure grade nitric acid was from J.T. Baker (Phillipsburg, NJ, USA). Analytical grade methanol and butanol were from Fisher Scientific (Fair Lawn, NJ, USA). Roundup, a commercial herbicide containing glyphosate and produced by Monsanto, was bought locally.

2.2. Preparation of analytical solution

Stock solutions (1 mg/ml) were prepared by weighing glyphosate and AMPA, and dissolving into Barnstead NANOpure water (Dubuque, IA, USA). When not in use, solutions were stored at 4 °C. Standard solutions and spiked samples were prepared by diluting the stock solutions with the mobile phase, the tap water or the lake water. The lake water was collected from the Campus Lake at Southern Illinois University–Carbondale, and filtered through 0.45-μm filters (Whatman, Clifton, NJ, USA).

2.3. Apparatus and analytical conditions

The construction of the laboratory-built CNLSD is given in several papers [17–19]. Like evaporative light scattering detection (ELSD), CNLSD is a universal, aerosol-based detection method, which detects the light scattering from the particles representing the non-volatile analytes in the mobile phase after solvent evaporation. Fig. 2 shows the conceptual difference between the detection process of ELSD and CNLSD. By a nebulizer, the eluent from

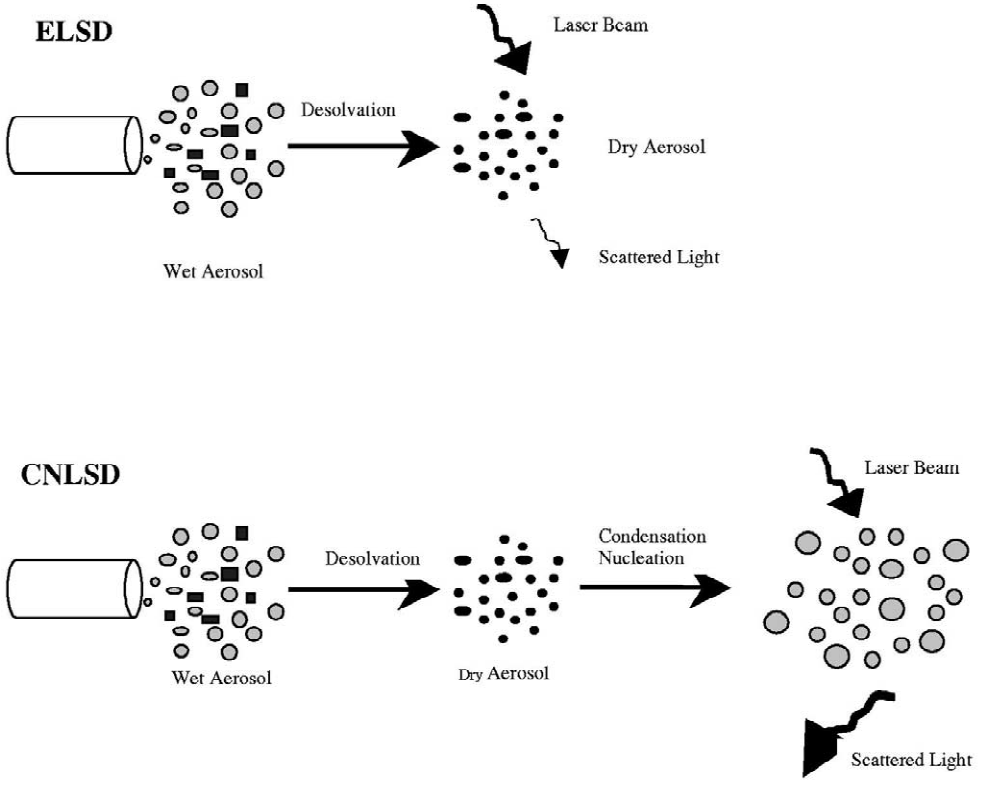

Fig. 2. Conceptual difference between the detection processes of ELSD and CNLSD.

the column is converted in both cases to wet aerosol droplets, which are then dried to form the dry aerosol particles. The light scattered directly by these particles is monitored with ELSD. However in CNLSD, a condensation nucleation process, which involves the growth of nanometer-sized particles to micrometer-sized droplets by condensation of an externally introduced vapor, is added. This increase in particle size tremendously increases the light scattering signal and dramatically increases the sensitivity in comparison to ELSD. A previous report [15] shows the LODs for CNLSD (~15 ng/ml) were about 130 times lower than those for a commercial ELSD for the detection of polyethylene glycols after separation by aqueous size exclusion chromatography.

The nonvolatile residue monitor (NRM) [25] is a commercial product developed by Fluid Measurement Technologies (FMT, Saint Paul, MN, USA), and it is used for continuously monitoring high-purity water in the semiconductor industry. NRM employs the same central processes, sample nebulization, aerosol drying and condensation nucleation, as CNLSD. A prototype CNLSD system (CNLSD using the NRM) was constructed based on an NRM apparatus [26] and used to perform the detection of the eluent from IC.

A Jasco 880-PU intelligent HPLC pump (Japan Spectroscopic, Tokyo, Japan) was used for the eluent delivery. The injection valve was a Rheodyne (Cotati, CA, USA) model 7125 with a 100-μl sample loop. Two 100×4.6 mm I.D. Alltech universal 7U cation-exchange columns (Deerfield, IL, USA) packed with polybutadiene–maleic acid-coated silica (7 μm) were connected in series to separate the analytes. The separation was performed at room temperature with 0.5 m*M* nitric acid as the eluent at the flow-rate of 0.5 ml/min. A home-written program was used to transfer and analyze the data collected by CNLSD.

3. Results and discussions

3.1. The influence of the mobile phase

Cation-exchange LC separations are recommended by the EPA [9] and popularly used [27,28] for the separation of glyphosate and AMPA. From our previous study [18], cation-exchange LC is more compatible with CNLSD than anion-exchange LC which gives higher background. So a cation-exchange column was chosen for this study.

Because CNLSD gives the response for all non-volatile compounds in the eluent, it is crucial to choose a reasonable mobile phase that provides low background and noise levels. LODs are closely related to the signal-to-noise ratio (S/N), so a pure and relatively volatile mobile phase has considerable advantage to reach good detection sensitivity for CNLSD. Previous work [18] found that nitric acid solutions are the most suitable mobile phase for IC–CNLSD analysis and its purity significantly affects the detection sensitivity.

The presence of organic solvent in the mobile phase has two effects on CNLSD. On one hand, it decreases the background level by lowering the surface tension of the mobile phase and making the desolvation process more efficient. On the other hand, organic solvents may increase the background intensity by introducing more non-volatile contaminants [17]. In our experiment, the latter is dominant. After adding 4% methanol into the mobile phase, the background level increased from 4000 to 10 000 counts/ml. Morris et al. [29] studied the effect of the alcohol modifiers on the separation of carboxylic acids using a cation-exchange LC column and proposed that the change of the separation should be attributed to the adsorption of a layer of alcohol on the polymeric resin surface. But here no significant change of retention behavior of the analytes was found, perhaps since we used much lower concentrations of methanol.

The pH value of the mobile phase scarcely affects the migration of glyphosate and AMPA. As shown in Fig. 1, glyphosate and AMPA are zwitterionic compounds, and the pH values will change the distributions of the structures of the analytes. Over the pH range 2–5, the pH range used in our experiment, the dominant forms are the species of the ion of charge −1, and 0 for glyphosate and AMPA, respectively. The pH value of the mobile phase may have two converse influences on the retention behavior of these compounds. Increasing the pH value of the mobile phase will decrease the eluting ability of the mobile phase, resulting in the longer retention time of the analytes [30]. On the other hand, a higher pH will also decrease the positive charges of the analytes

and make the retention time shorter. As a result, little change of the retention time of these analytes was observed as a function of pH.

Because CNLSD responds to all the non-volatile species, non-volatile contaminants present in the mobile phase will increase the background and limit the sensitivity. Though high-purity nitric acid was used as the mobile phase, some heavy metal ion contaminants may be present [18]. Low acid concentrations lead to low contaminant levels, so a low nitric acid eluent concentration is preferable for CNLSD detection. As a result, 0.5 m*M* HNO_3 was chosen as the mobile phase.

Diffusion screens are useful to reduce the background in CNLSD [15]. Diffusion screens are fine mesh screens to which smaller particles, having higher diffusion coefficients, will be collected before the condensation nucleation process. Because the signals of the analytes also decrease along with the background, optimization of the number of the diffusion screens is generally needed with CNLSD. Results showed that two diffusion screens worked well to lower the background level without significantly changing the response of the analytes.

Due to their amphoteric nature, the sample pH value will affect the charges of glyphosate and AMPA, which produce different retention times with the same mobile phase. With the increase of sample pH, the retention times of the analytes decrease. A pH value of 3, the same pH value of the mobile phase, was used in our experiments.

3.2. The influence of the flow-rate of the mobile phase

A low flow-rate is beneficial to the ion-exchange process. Because the dominant forms of glyphosate and AMPA in the pH range used are of negative charges, they are less retentive on the cation-exchange column. So a lower flow-rate can be used to increase the detection times of these species. As shown in Fig. 3 for a spiked lake water sample, two peaks may affect the detection of the analytes. Results showed that when the flow-rate was reduced to 0.5 ml/min, all four peaks were reasonably well-separated. No common cations, such as metal ions, interfere because of their relatively longer retention times.

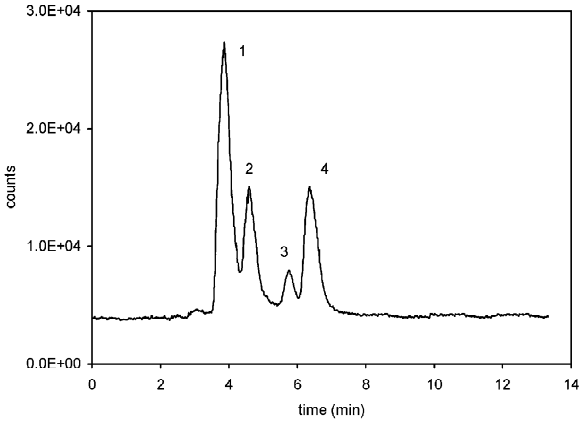

Fig. 3. Chromatogram of the spiked analytes in lake water. Conditions: Alltech universal 7U cation-exchange column, 0.5 m*M* HNO_3 as the eluent as the flow-rate of 0.5 ml/min, 18 p.s.i. of air pressure and two diffusion screens. Peaks: 1 and 3= interference peaks; 2=glyphosate (3.03 μg/ml); 4=AMPA (4.32 μg/ml).

3.3. Influence of the air pressure

Air was used as the nebulizing gas in the CNLSD with NRM system, and its pressure, influencing the gas flow-rate, is an important factor to affect the performance of CNLSD. Fig. 4 shows the influence of air pressure on the detection at two different flow-rates of the mobile phase. These data demonstrate that a higher flow-rate of the mobile phase would require a higher air pressure to obtain the optimum *S*/*N*. With the decrease of the air pressure, the background and noise level increased. This may be the result of reduction of the transport efficiency of the aerosol. In CNLSD using the NRM system, the air flow-rate is much higher than that in the conventional CNLSD system and no heating is needed for desolvation. Here, the air is used not only to produce the aerosol but also to dry the aerosol. Because the analyte particle concentration was diluted by the higher flow-rate air, the signal in CNLSD using the NRM system is relatively lower than that in the laboratory-made CNLSD system [16–19]. However, the background and noise are also lower, so little difference of *S*/*N* and LOD was observed between the two systems. With CNLSD using the NRM system, the LODs are about 50 μg/ml (Table 1) at a background level about 3800

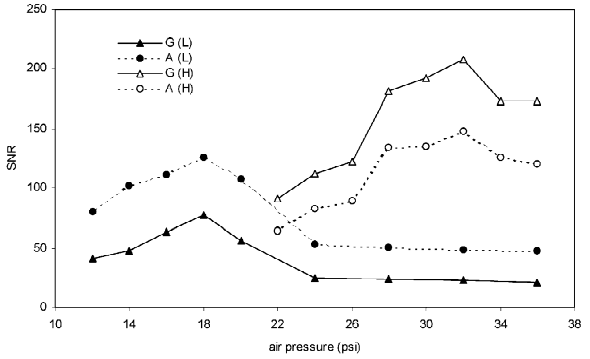

Fig. 4. The influence of the air pressure on CNLSD sensitivity: glyphosate (G) and AMPA (A) at the mobile phase flow-rate of 0.5 ml/min (L) and at the mobile phase flow-rate of 1.0 ml/min (H).

counts/ml. With the laboratory-made CNLSD system, the LOD changed little (54 and 58 ng/ml for glyphosate and AMPA, respectively) though the background level was higher and about 8200 counts/ml.

3.4. Analysis of glyphosate and AMPA in different matrix

At the optimized conditions, with 0.5 m*M* HNO_3 as the mobile phase at the flow-rate of 0.5 ml/min, two diffusion screens, and 18 p.s.i. of air pressure, glyphosate and AMPA were analyzed in different matrixes (1 p.s.i.=6894.76 Pa). None of the analytes were detected in the tap water or lake water.

The performance difference between the prototype CNLSD and the laboratory-built CNLSD system is listed in Table 1. It follows from Table 1 that calibrations for the two analytes have very good linearity (R^2=0.999) over two orders of magnitude. The LODs calculated (~50 ng/ml; S/N=3) are much lower than the MCL of 700 ng/ml for glyphosate in drinking water issued by the EPA and, therefore, this method can be used directly for water quality control without preconcentration. It should be noted that the European Union has stricter regulation for pesticides in water such that the maximum concentration of a pesticide should not exceed 0.1 ng/ml [31]. For this case, a sample preconcentration would be required. The preconcentration methods for glyphosate in water were reviewed [3]. However, taking into account the fact that the toxicity of glyphosate is very low for humans, the result should be considered satisfactory.

The analytes at the different concentrations (0.20–2.88 μg/ml) were spiked into deionized water, tap water and lake water. Table 2 is the results of the mean recoveries and relative standard deviations (RSD) for these different matrixes. The recoveries ranged from 92.9 to 108.7%, while the RSD was lower than 5% in all cases. The average recovery for the entire data set was 100±3.6%, indicating accurate quantization for the method. The average RSD for the data set was 2.5%, indicating good reproducibility for the method.

A commercial herbicide containing glyphosate, distributed by Monsanto as Roundup, was also

Table 1
Performance difference between the prototype CNLSD system and the laboratory-built CNLSD system

Method		Prototype	Laboratory-made
t_R (min)	Glyphosate	4.6	4.2
	AMPA	6.4	5.8
LOD (ng/ml)	Glyphosate	41	54
	AMPA	53	58
Linearity range (ng/ml)	Glyphosate	41–3030	54–4000
	AMPA	53–4320	58–5040
Calibration curve	Glyphosate	$y = 3748.1x + 148.73$	$y = 7878.8x + 2468.1$
	AMPA	$y = 2941.5x + 591.03$	$y = 7399.4x + 2396.5$
R^2	Glyphosate	0.9984	0.9993
	AMPA	0.9986	0.9990
Noise level		50	140
Background level		3800	8200

Table 2
Mean recovery and relative standard derivation (RSD) for the analytes at different concentrations in different matrices

Matrix		Concentration (μg/ml)	Mean recovery (%)	RSD (%, $n=6$)
DW	Glyphosate	0.20	99.0	2.8
		0.71	101.0	2.7
		1.01	100.9	2.1
		2.02	96.0	1.5
	AMPA	0.29	92.9	4.9
		1.01	101.2	2.1
		1.44	104.1	0.6
		2.88	100.3	2.5
TW	Glyphosate	0.20	95.9	4.6
		0.71	105.8	1.3
		1.00	98.2	2.2
		2.00	102.6	0.8
	AMPA	0.25	108.7	3.4
		0.88	95.6	4.0
		1.26	96.6	3.2
		2.52	101.3	1.1
LW	Glyphosate	0.20	93.1	3.6
		0.71	107.4	1.4
		1.01	97.7	2.5
		2.02	100.4	1.9
	AMPA	0.29	92.9	3.3
		1.01	102.4	2.9
		1.44	104.1	2.6
		2.88	102.9	2.7
	Average		100.0±3.6	2.5

DW = deionized water; TW = tap water; LW = lake water.

analyzed by this method. There were no additional sample preparation steps except for diluting the solution 50 000-fold with the mobile phase. No interference peak was found and the results obtained showed an RSD of 1.1% for six injections. The signal level predicts a concentration level of 10.7% in the sample solution compared to 12.5% indicated on the label.

4. Conclusions

The IC–CNLSD approach has been developed successfully for the analysis of a polar pesticide glyphosate and its metabolite AMPA. Compared to other reported methods, the IC–CNLSD method has no need of sample derivatization, preconcentration, or mobile phase conductivity suppression. The method is simple, fast and inexpensive. Results show CNLSD is an ideal direct detection technique for such pesticides without chromophores or fluorophores when present in the environmental samples.

CNLSD is a new detection method that will be soon commercially available [26]. This study also shows that the prototype CNLSD system has comparable performance to the laboratory-built CNLSD system and that a commercial version of CNLSD based on an NRM is feasible.

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