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Analysis of biogenic amines using corona discharge ion mobility spectrometry

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ABSTRACT

A new method based on corona discharge ion mobility spectrometry (CD-IMS) was developed for the analysis of biogenic amines including spermidine, spermine, putrescine, and cadaverine. The ion mobility spectra of the compounds were obtained with and without n-Nonylamine used as the reagent gas. The high proton affinity of n-Nonylamine prevented ion formation from compounds with a proton affinity lower than that of n-Nonylamine and, therefore, enhanced its selectivity. It was also realized that the ion mobility spectrum of n-Nonylamine varied with its concentration. A sample injection port of a gas chromatograph was modified and used as the sample introduction system into the CD-IMS. The detection limits, dynamic ranges, and analytical parameters of the compounds with and without using the reagent gas were obtained. The detection limits and dynamic ranges of the compounds were about 2 ng and 2 orders of magnitude, respectively. The wide dynamic range of CD-IMS originates from the high current of the corona discharge. The results revealed the high capability of the CD-IMS for the analysis of biogenic amines.

1. Introduction

Biogenic amines are a class of small molecules that are produced and metabolized *in vivo*. They play an important role in human pathology and physiology and serve as biochemical markers for cancer, diabetes, arthritis and cystic fibrosis [1]. It is well-known that the putrescine, spermidine, spermine and cadaverine have close relationships with malignant tumors [2]. In addition, the high concentration level of biogenic amines in foods, which is formed by microbial decarboxylation of amino acids, implies their deterioration [3]. The chemical formula of biogenic amines used in this work is shown in Fig. 1.

Different methods have been developed for the analysis of biogenic amines in serum and body fluids including fluorescence, ELISA, high performance liquid chromatography (HPLC) [4–8], gas chromatography (GC) [9–11], GC–MS [12], enzymatic sensor [13], HPLC with conductometric [14], and capillary zone electrophoresis with amperometric detection [15], or capillary electrophoresis with electrochemiluminescence detections [16]. Chromatographic techniques require derivatization of the amines, because highly polar amines tend to "stick" to the column and cause the "memory effect" [17]. In addition, IMS with ^{63}Ni as the ionization source and n-Nonylamine as the reagent gas have been used by Karpas et al. [17,18] for the analysis of biogenic amines in vaginal fluid and in meat food products. Gas chromatography–differential mobility spectrometry has also been reportedly used for the analysis of putrescine and cadeverine [19].

IMS is a sensitive, simple, fast, and portable analytical instrument for the detection of volatile organic compounds (VOCs) at low concentrations. A key element of the instrument is the ionization source, for which ^{63}Ni is typically used. Corona discharge (CD) is an alternative ionization source for IMS with several advantages over ^{63}Ni. The CD is a non-radioactive source, which is capable of producing a current one order of magnitude higher than ^{63}Ni. This results in a better sensitivity, higher signal-to-noise ratio, and a wider dynamic range [20]. IMS can be performed in either positive or negative mode. In the positive mode, the analyte is ionized in ion-molecular exchange reactions with reactant ions, which are commonly hydrated protons. In order to remove background interferences and enhance sensitivity, reagent gases may be used to create alternate reactant ions that provide enhanced selectivity in the response.

The objective of this study was to evaluate the potential of CD-IMS for direct quantitative analysis of biogenic amines using n-Nonylamine as the alternate reagent gas. For the purposes of this study, a sample introduction system was developed with unique characteristics, which is a modified version of the sample injection port of a GC.

2. Experimental

2.1. Instrumentation

The IMS apparatus with the corona discharge ionization source in the positive mode has been described previously [21,22]. The schematic diagram of the IMS cell is shown in Fig. 2a. In this work, the sample gas was introduced into the cell via a brass tube (i.d. 3 mm and o.d. 6 mm), positioned orthogonal to the corona nee-

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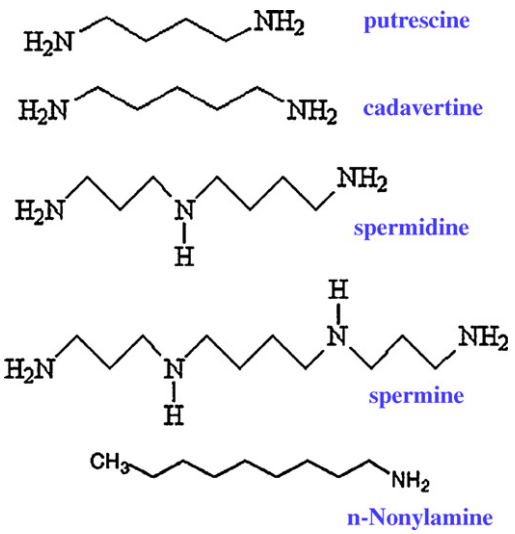

Fig. 1. Chemical formula of the aliphatic biogenic amines investigated.

dle. This tube was also used as the counter electrode [23]. The brass tube and an aluminum ring around the IMS cell were connected to ensure identical potentials. Nitrogen was used as the drift gas, carrier gas and purging gas. n-Nonylamine was doped with the drift gas. The injection port for introducing liquid samples into the IMS cell has been described elsewhere [24]. In this work, the sample introduction system was a modified injection port of a gas chromatograph, as shown in Fig. 2b. This system consists of a cubic shape of 60 mm × 50 mm × 25 mm dimensions. A cartridge heater, 250 watt, was used for heating the port up to 200 °C and a micro-syringe for injecting the sample through a septum into the injection port. Carrier gas inlet and outlet were placed opposite to each other (Fig. 2b). The optimized experimental conditions for obtaining the ion mobility spectra of the compounds are given in Table 1.

2.2. Materials and methods

Methanol as the solvent and n-Nonylamine as the reagent gas were purchased from Merck. The biogenic amines including spermine, spermidine, putrescine and cadaverine were purchased

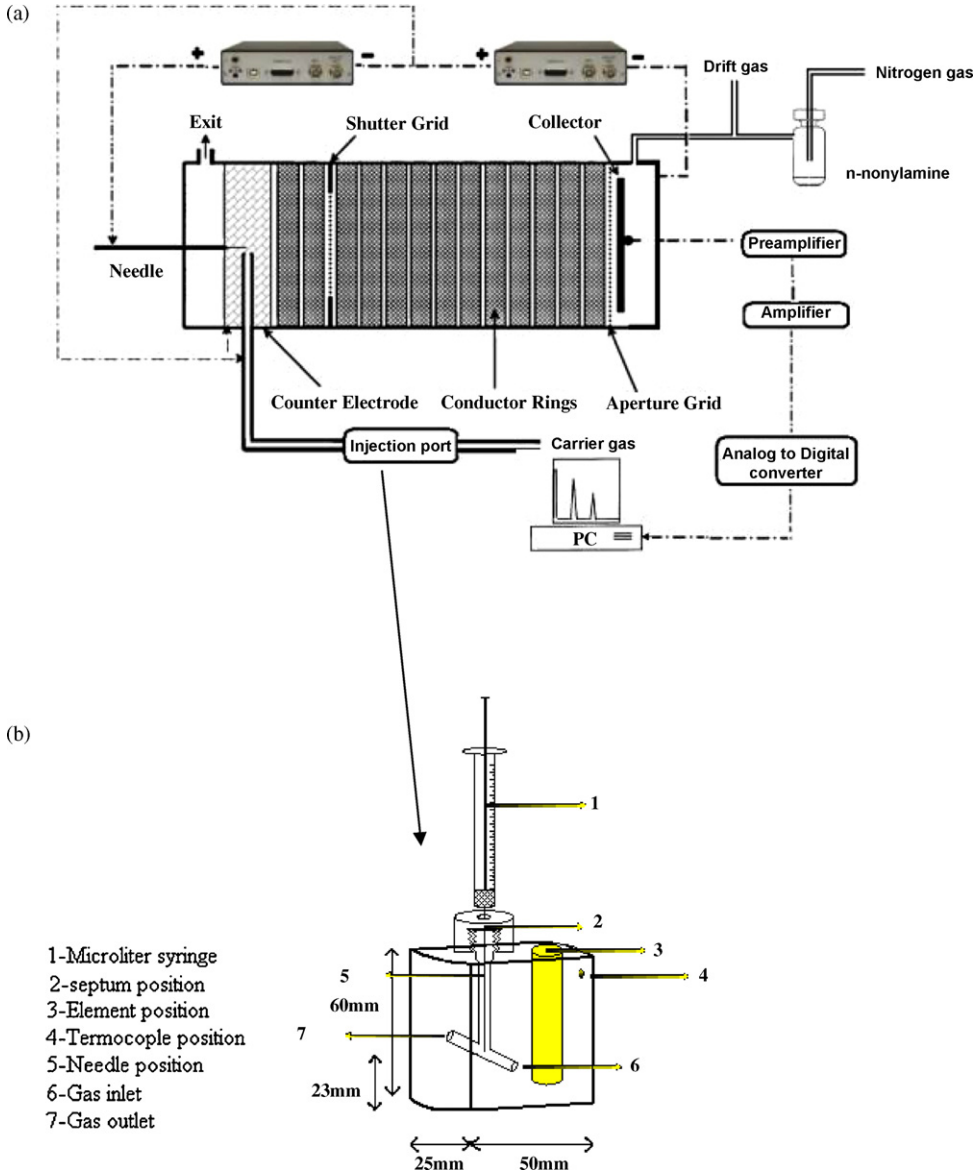

Fig. 2. Schematic diagram of the CD-IMS with the sample introduction system used in this work: (a) CD-IMS and (b) sample introduction system.

Table 1
Summary of the parameters used in this study.

Operating parameters	Setting
Corona voltage	12.00 kV
Counter electrode voltage	8.00 kV
Drift flow gas (N_2)	380 mL min^{-1}
Carrier flow gas (N_2)	270 mL min^{-1}
N_2 flow rate for doping	40 mL min^{-1}
Cell temperature	175 °C
Injection port temperature	180 °C
Shutter grid pulse	100 μs
Drift field	533 V cm^{-1}
Reagent gas	$C_9H_{21}N$

(≥99%) from Fluka. Stock standard solutions (100 μg mL^{-1}) from each biogenic amine were prepared in N_2 atmosphere because they are sensitive to carbon dioxide. Working solutions were prepared through successive dilutions of the stock solutions.

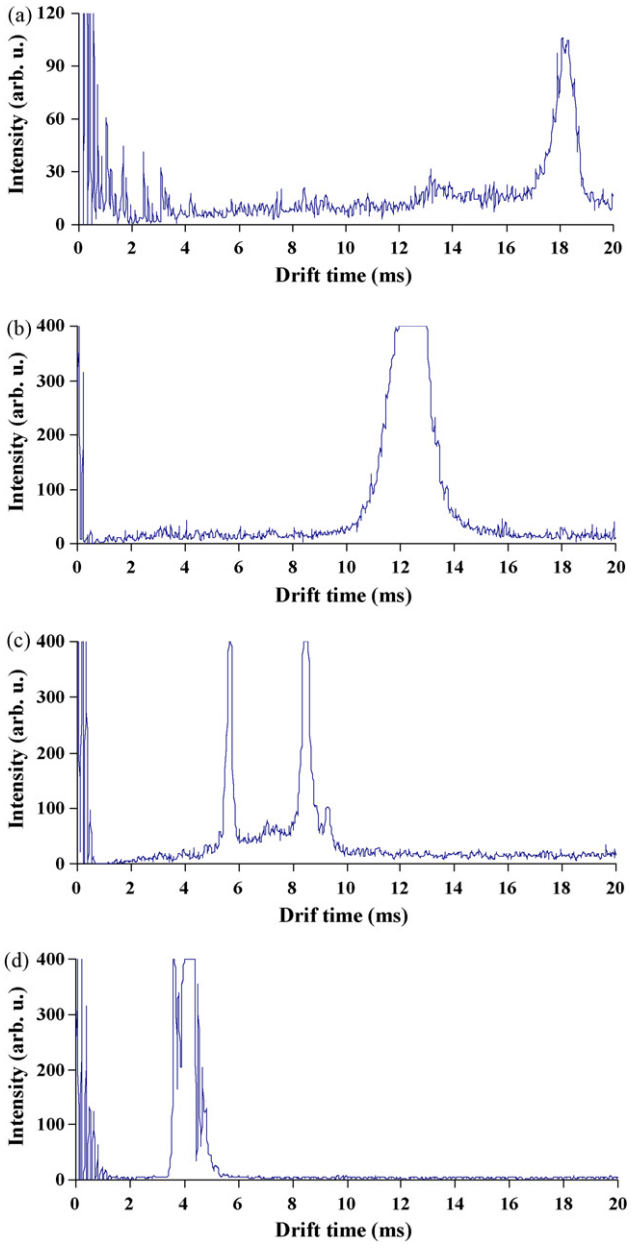

Fig. 3. Ion mobility spectra of the reagent gas (n-Nonylamine) at different mass flow rates.

3. Results and discussion

3.1. Sample introduction system

In this work, a specially designed sample introduction system (Fig. 2b) was developed to prevent peak broadening or reduced

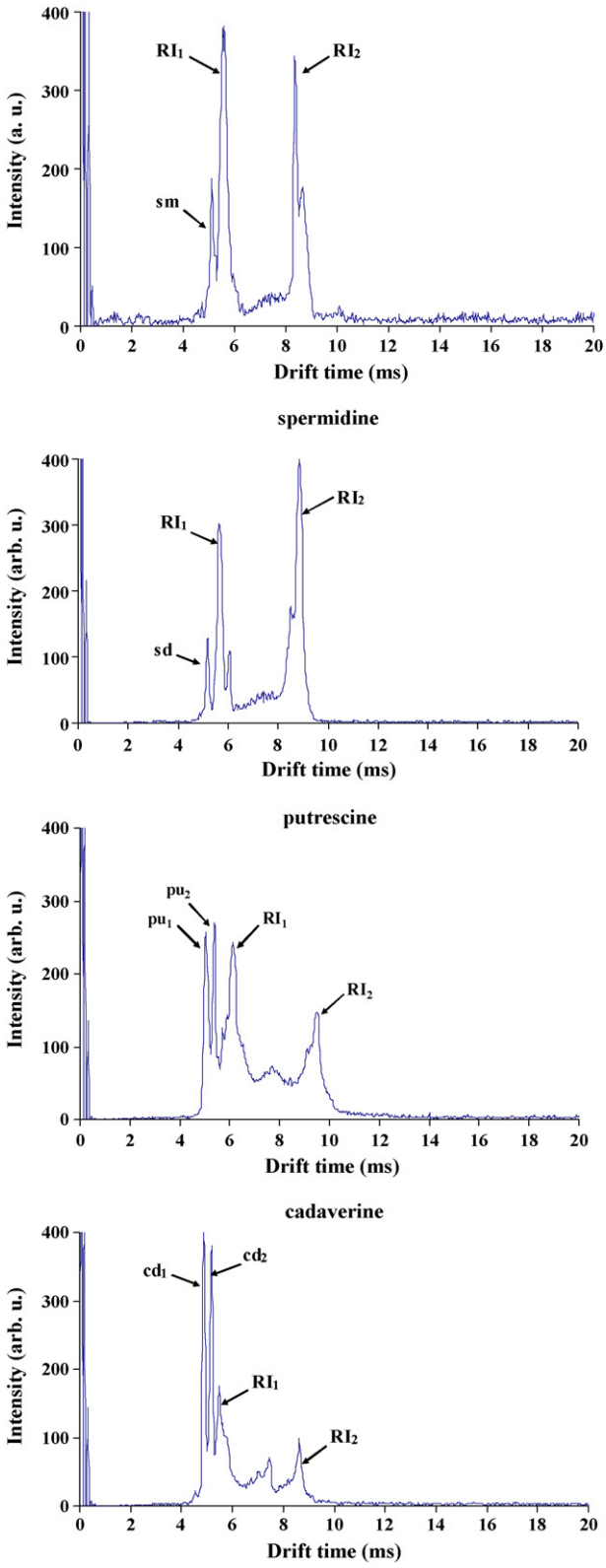

Fig. 4. Ion mobility spectra of spermine, spermidine, putrescine and cadaverine using n-Nonylamine as the reagent gas.

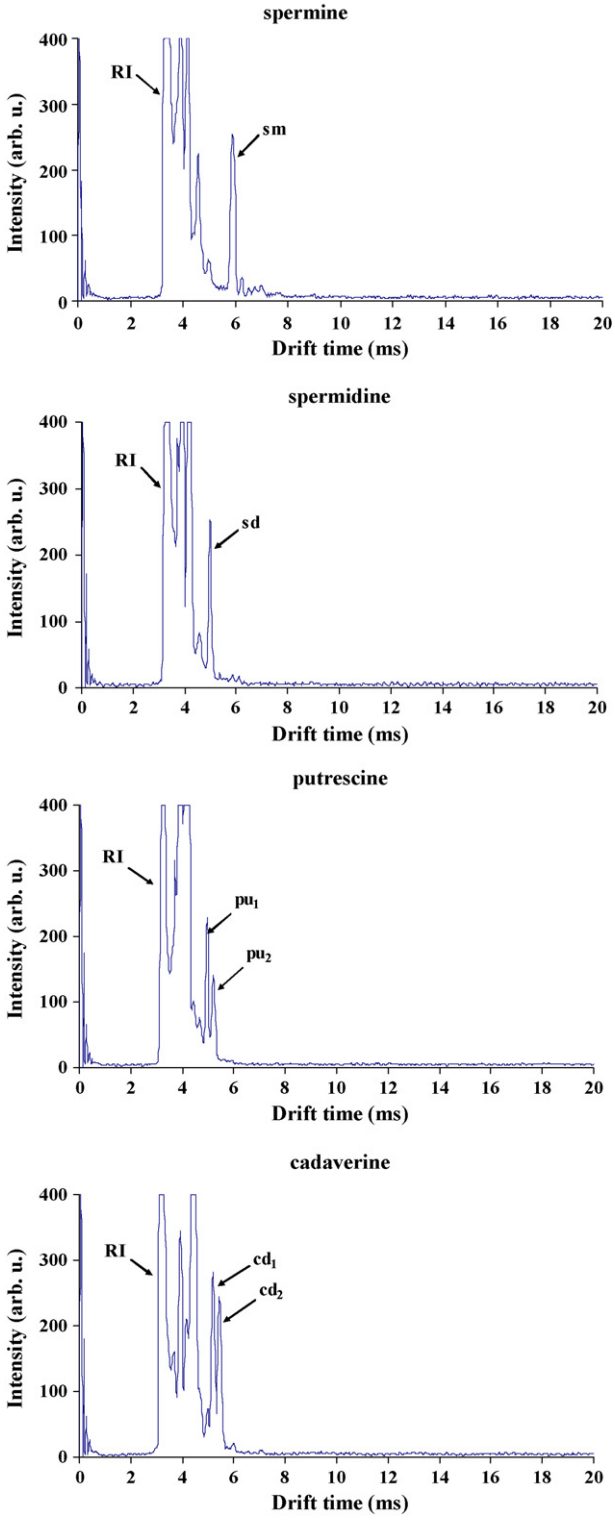

Fig. 5. Ion mobility spectra of spermidine and spermidine, putrescine and cadaverine without using n-Nonylamine as the reagent gas.

resolution of the method. The diameter of the injection port for inserting the syringe needle and the volume of the sampling chamber were reduced to the smallest caliber possible. When the syringe needle was inserted into the injection port, its tip was located in the sampling chamber. The inlet and outlet of the carrier gases were positioned on opposite sides of the sampling chamber. This modified injection port was thus able to conveniently introduce the sample into the CD-IMS.

3.2. n-Nonylamine as the reagent gas

n-Nonylamine as the reagent gas was doped with the drift gas. Karpas et al. [17,18] have reported the advantages of the very high proton affinity (219 kcal mol^{-1}) of n-Nonylamine as the reagent gas for the analysis of biogenic amines. This property of n-Nonylamine prevents ion formation of compounds with proton affinities lower than that of n-Nonylamine, thus, eliminating spectral interferences from many compounds. It should be mentioned that proton affinity of n-Nonylamine is lower than those of biogenic amines and, therefore, biogenic amines are capable of capturing protons from n-Nonylamine clusters. Another advantage of n-Nonylamine used in this work concerns its higher proton affinity compared with methanol (185 kcal mol^{-1}) used as the solvent. Therefore, methanol could not capture proton from n-Nonylamine and, thus, did not contribute to spectral interferences.

n-Nonylamine was doped with the drift gas at different mass flow rates, the results of which are shown in Fig. 3. It is worth noting that ion formation of n-Nonylamine clusters in IMS and its ion mobility spectrum changed in proportion to its mass flow rate. The mass flow rate of n-Nonylamine was obtained on the basis of vaporization enthalpy, ΔH^g of n-Nonylamine, vapor pressure of the reagent at the doping temperature (25 °C using Clausius–Clapeyron equation) and N_2 flow rate, used for purging n-Nonylamine. A simple and convenient method for estimating the vaporization enthalpy has been suggested by Hildebrand [25]. In this empirical equation, ΔH^g is calculated in terms of the absolute boiling temperature T_b (at 760 mmHg):

$$\Delta H^g(298)/\text{cal mol}^{-1} = -2950 + 23.7\,T_b + 0.02\,T_b^2 \quad (1)$$

As n-Nonylamine has a boiling point of 202 °C, its calculated ΔH_g and mass flow rate are 12.8 kcal mol^{-1} and 5.38×10^{-7} mol min^{-1} (40 mL min^{-1} N_2 for purging of n-Nonylamine), respectively. The ion mobility of n-Nonylamine at this mass flow rate is shown in Fig. 3c. This mass flow rate of doping was the optimized value and the ion mobility spectra of the biogenic amines were obtained under these experimental conditions. At this mass flow rate, two separate ion clusters formed with reduced mobilities of 1.64 and 2.61 $cm^2\,V^{-1}\,s^{-1}$. The former, $K_0 = 1.64 \pm 0.019\,cm^2\,V^{-1}\,s^{-1}$, is very close to the value reported by Karpas et al. [17]. The latter with a lower drift time or a higher reduced mobility is probably due

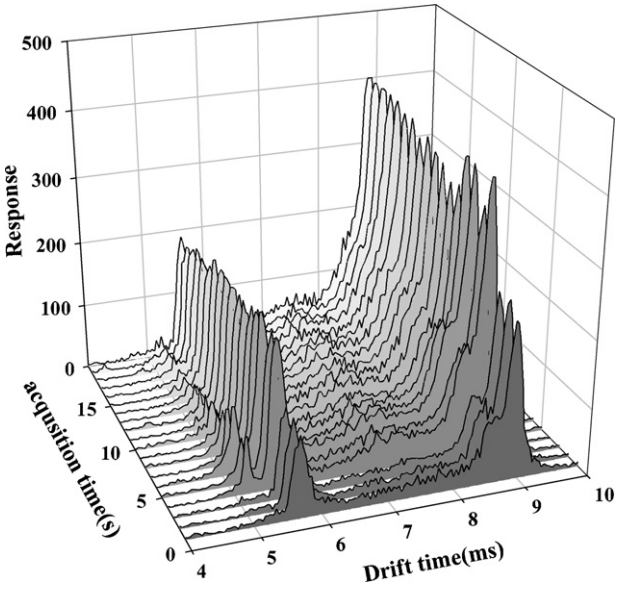

Fig. 6. A 3D plot of the ion mobility spectra of spermidine.

to a fragment of n-Nonylamine. At n-Nonylamine mass flow rates larger than this value, the ion clusters appeared at higher drift times (Fig. 3a and b). In such cases, the proton affinity of n-Nonylamine clusters and their concentrations were too high for the analytes to be able to capture protons from the clusters. Therefore, when biogenic amines were injected into the cell, no peak was observed from the sample. Finally, protonated water and methanol clusters formed without doping n-Nonylamine (Fig. 3d).

3.3. Ion mobility spectra

The ion mobility spectra of spermidine, spermine, putrescine, and cadaverine using n-Nonylamine as the reagent gas are shown in Fig. 4 These spectra were obtained under the optimized IMS conditions given in Table 1. The reduced mobility of the product ions of these compounds is listed in Table 2. It should be mentioned that no report was found in the literature on reduced mobilities of these compounds. In order to identify these ion clusters, it was, therefore, necessary to couple the IMS with a mass spectrometer. However, the reduced mobility of the ions was calculated based on the ion reduced mobility of n-Nonylamine at 1.63 $cm^2\ V^{-1}\ s^{-1}$ as reported by Karpas et al. [17]. These reduced mobility values could then be used for comparison of ion mobility spectra and for ion identification.

In this work, the ion mobility spectra of the biogenic amines without using n-Nonylamine were also obtained and reported in Fig. 5. The reduced mobility values of the ions were calculated based on the reduced mobility of nicotinamide (1.85 $cm^2\ V^{-1}\ s^{-1}$) [26]. The ion mobility spectra contain the ions originating from methanol injected into the sample introduction system as the solvent. The main limitation in the analysis of biogenic amines under these conditions stems from the possibility of spectral interference from any compound with a higher proton affinity than water and methanol.

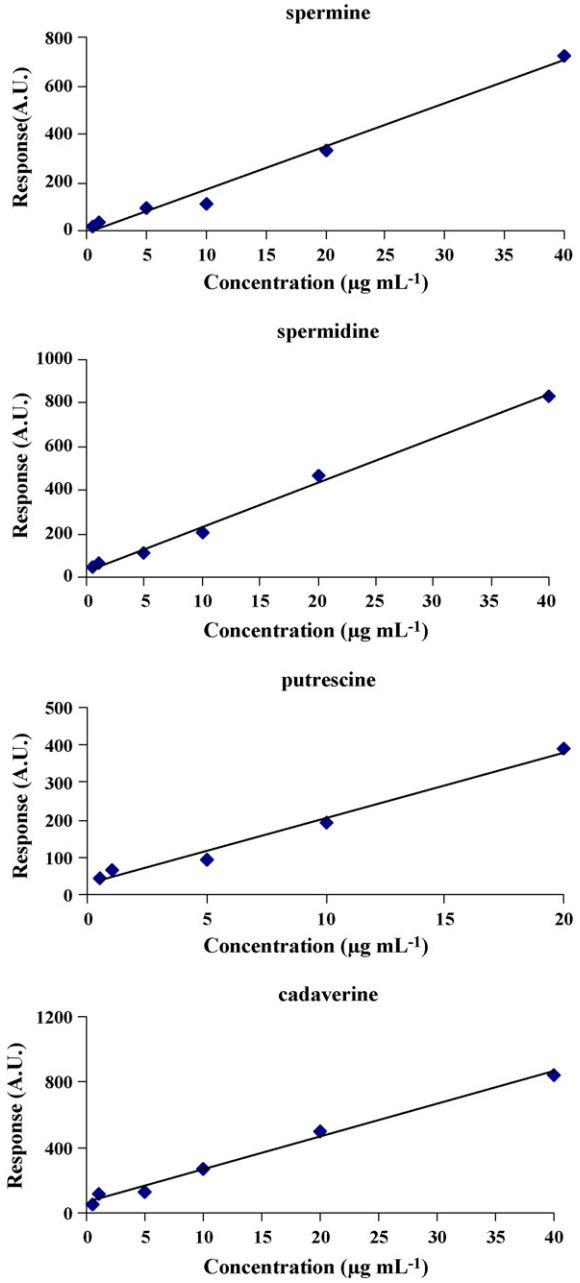

Fig. 7. Calibration plots of the biogenic amines using n-Nonylamine as the reagent gas.

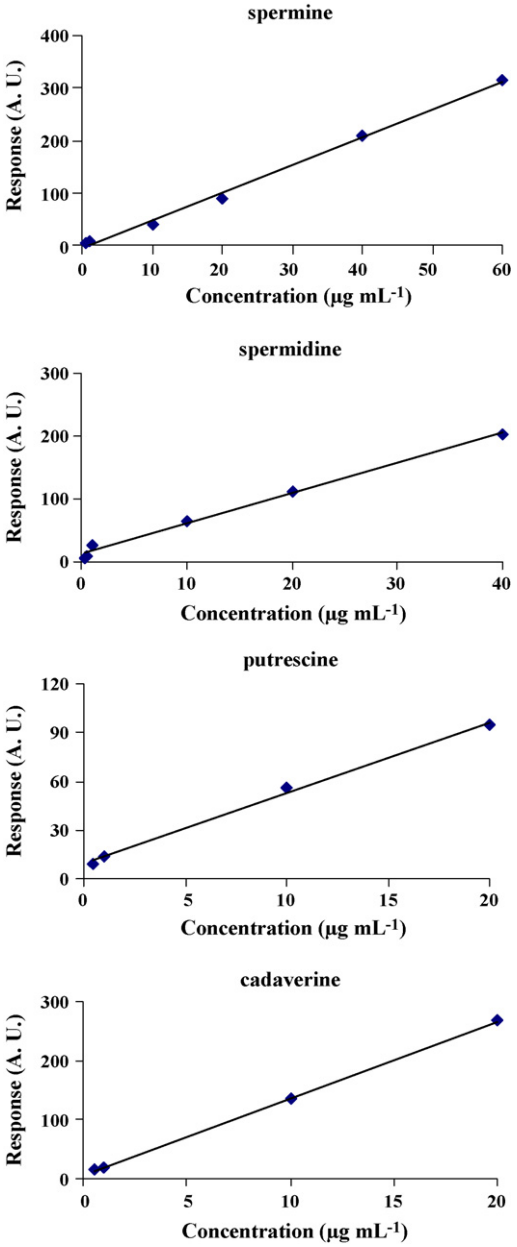

Fig. 8. Calibration plots of the biogenic amines without using n-Nonylamine as the reagent gas.

Table 2
Drift time and reduced mobility values (K_0) for the produced ions with and without n-Nonylamine used as the reagent gas.

With using n-Nonylamine			Without using n-Nonylamine		
Peak	Drift time (ms)	K ($cm^2\ V^{-1}\ s^{-1}$)	Peak	Drift time (ms)	K ($cm^2\ V^{-1}\ s^{-1}$)
RI_1	5.60	2.61 ± 0.03	sm	5.88	2.83 ± 0.03
RI_2	8.92	1.64 ± 0.02	sd	4.96	3.35 ± 0.03
sm	5.16	2.83 ± 0.03	pu_1	4.96	3.35 ± 0.03
sd	5.20	2.81 ± 0.03	pu_2	5.20	3.20 ± 0.03
pu_1	4.72	3.09 ± 0.04	cd_1	5.20	3.20 ± 0.03
pu_2	5.04	2.90 ± 0.03	cd_2	5.44	3.06 ± 0.03
cd_1	5.08	2.87 ± 0.03			
cd_2	5.20	2.81 ± 0.03			

RI_1: The first reactant ion.
RI_2: The second reactant ion.
sm: The peak of spermine.
sd: The peak of spermidine.
pu_1: The first peak of putrescine.
pu_2: The second peak of putrescine.
cd_1: The first peak of cadaverine.
cd_2: The second peak of cadaverine.

Table 3
Analytical parameters for the compounds with and without n-Nonylamine used as the reagent gas.

With using n-Nonylamine				Without using n-Nonylamine		
Analyte	Relative standard deviation (RSD)	Correlation coefficient (R)	Detection limit (DL)	Relative standard deviation (RSD)	Correlation coefficient (R)	Detection limit (DL)
Spermine	12	0.992	1.9 ng	10	0.998	2.5 ng
Spermidine	11	0.997	1.7 ng	11	0.996	2.7 ng
Cadaverine	13	0.995	1.7 ng	12	0.999	3.1 ng
Putrescine	10	0.992	2.0 ng	10	0.998	1.0 ng

Since the proton affinity of methanol and water are too low, many compounds could cause spectral interferences with the analysis of the biogenic amines.

3.4. Calibration curves and analytical parameters

The watershed 3D plots of the ion mobility spectra of spermidine with a concentration of 10 $\mu g\ mL^{-1}$ using n-Nonylamine are shown in Fig. 6, indicating that the relative peak intensities change during the acquisition time. The product ion peaks, which appear after a short period of time, reach a maximum which is followed by an almost exponential decay. The area under each peak was calculated during the acquisition time (starting from the injection time to the disappearance of the sample peaks). Then, the sum of the areas is plotted versus acquisition time. Integration of this plot is considered as the response. The calibration curve is obtained by plotting the response against the concentration of the compounds. The calibration plots for different amines are shown in Fig. 7, and the corresponding analytical parameters are listed in Table 3. As can be seen, the correlation coefficients of the calibration plots are between 0.992 and 0.997. The detection limit for all the compounds was about 2 ng and the linear dynamic range was 2 orders of magnitude. The relative standard deviation (RSD%) was found to be 12%. Comparison of the dynamic ranges of the proposed method (2–400 ng) and the reported values for putrescine and cadaverine (3–20 ng) [19], using thermal desorption gas chromatography–differential mobility spectrometry, reveals the potential of the proposed method for the analysis of biogenic amines.

Calibration plots of the biogenic amines without using n-Nonylamine are shown in Fig. 8, and the corresponding analytical parameters are listed in Table 3. The results indicate that the detection limits and dynamic ranges of the compounds in this condition are approximately similar to the previous case in which n-Nonylamine was used.

4. Conclusion

Several biogenic amines were analyzed with IMS using corona discharge as an ionization source. The ion mobility spectra, calibration curves and analytical parameters of the compounds were obtained with and without using n-Nonylamine as the reagent gas. The results indicate that it would be possible to analyze the biogenic amines without using n-Nonylamine as the reagent gas. However, there is a possibility for spectral interference from any compound with the higher proton affinity than water and methanol (used in this work). The proposed method is a derivative free, fast and simple technique for the analysis of biogenic amines. The dynamic ranges of the compounds reported in this work are much wider than those reported previously using the IMS method. It was also found that the ion mobility spectra of n-Nonylamine changed with its concentration. In addition, an injection port of a gas chromatograph was modified to be used as an introduction system for the CD-IMS.

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