Published in final edited form as:

Anal Chim Acta. 2010 January 18; 658(1): 91–97. doi:10.1016/j.aca.2009.10.058.

Ribonucleotide and Ribonucleoside Determination by Ambient Pressure Ion Mobility Spectrometry (IMS)

Abu B. Kanu¹, Greg Hampikian², Simon D. Brandt³, and Herbert H. Hill Jr.^{1,*}

¹ Department of Chemistry, Washington State University, Pullman, WA 99164-4630, USA

² Department of Biology & Criminal Justice Administration, Boise State University, Boise, ID 83725-1515

³ School of Pharmacy & Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK

Abstract

Reduced mobilities, resolving powers and detection limits for 12 ribonucleotides and 4 ribonucleosides were measured by ambient pressure electrospray ionization ion mobility spectrometry (ESI-IMS). With the instrument used in this study it was possible to separate some of these compounds within the mixtures. In addition, the detection limits reported for the ribonucleotides and ribonucleosides ranged from 15 to 300 picomoles whereas resolving power ranged from 41 to 56 suggesting that ambient pressure ESI-IMS may be used for their rapid and sensitive separation and detection. This short report demonstrates that it was possible to use IMS for the separation of nucleotides and nucleosides in less than one second. The application holds great promise for nucleotide analysis in the area of separating DNA fragments in genome sequencing and also for forensics DNA typing examinations used for the identification of blood stains in crime scenes and paternity testing.

Keywords

Electrospray ionization; Ion mobility spectrometry; Nucleotides; Detection limit; Resolving power

Introduction

Nucleotides form the structural basis of life and are chemically characterized by the presence of three structural features (a nitrogenous base, a sugar, and a phosphate component). In recent years, nucleotide analysis has become increasingly important for the understanding of processes involved, for example, in cell formation, replacement, development of immune and sperm cells and mechanisms associated with the reproduction of the female tract. Because nucleotides are the building blocks of DNA and RNA molecules, many strategies have been used for their analysis [1–3]. One of the most commonly used instrumental approaches is based on mass spectrometry [4–6]. In addition to mass spectrometry, several other research groups have utilized capillary electrophoresis (CE) and high performance liquid chromatography (HPLC). The use of CE has been described for the separation of polymerase chain reaction

*Corresponding Author: Ph: 509-335-5648; Fax: 509-335-8867. hhhill@wsu.edu (H.H. Hill Jr.); abbkanu@gmail.com (A.B. Kanu).

This work is currently developing a method for the rapid and sensitive detection and separation for ribonucleotides and ribonucleosides. This technique if successful developed, holds great promise for nucleotide analysis in the area of separating DNA fragments in genome sequencing and also for forensic DNA typing examinations used for identification of blood stains in crime scenes and paternity testing. We present our preliminary data from this investigation.

(PCR) products in combination with electrokinetic injection where a narrow band of sieved DNAs was introduced through various entangled polymer matrices. Detection was carried out via single or multichannel laser-induced fluorescence [7]. Other studies have developed CE methods for gene mutation detection in the following areas: (1) single-strand conformation polymorphism with capillary electrophoresis; (2) SNaPshot analysis; (3) constant denaturant capillary electrophoresis; (4) microsatellite analysis; and (5) methylation analysis [8].

Most cancers have been linked to mutations within specific nucleotide DNA sequences that code for critically important bio-molecules such as tumor suppression proteins. Unambiguous identification of these DNA reaction sites requires the implementation of suitable methods for the accurate analysis of oligonucleotide fragments derived from strand breakage that may occur at specific sites. A capillary electrophoresis laser induced fluorescence (CE-LID) assay was reported for analysis of DNA fragment sizing [9]. A CE-LID assay has also been developed for effective DNA extraction, reflecting the increased need for miniaturization and automation applied to genetic analysis [10]. In addition to these developments, several other methods have been reported. These included nucleotide sequence variation analysis of human mitochondrial DNA molecules [11], analysis of DNA nucleotides and nucleosides involved in platinum-based anticancer therapy using capillary electrophoresis electrospray ionization mass spectrometry (CE-ESI-MS) [12], application of a CE-LIF assay for the detection of adenylyl cyclase activity [13], microchip affinity CE for studying protein DNA-binding on a borofloat glass microchip using thrombin and a fluorescently labeled thrombin-binding aptamer [14], multiplex-PCR CE for genotyping closely related isolates of four-locus single nucleotide repeats [15] and micro-CE chip for high-throughput analysis of bio-samples [16], short-end injection capillary zone electrophoresis (CZE) for evaluating methylcystosine/total cystosine ratio after acidic DNA analysis [17]. Other studies investigating either nucleotides or nucleosides in different matrices have utilized tandem techniques such as CE-ESI-MS [18], LC-ICP-MS [19], LC positive ion ESI-MS-MS [20], ESI-HPLC-MS-MS [21], LC-LC-ESI-MS [22], ion pairing HPLC-ESI-MS-MS [23], and pressure assisted CE-MS [24]. All of these investigations accentuate the importance of developing a rapid and sensitive method for the analysis of nucleotides.

Of the instruments that have been applied to nucleotide and nucleosides analysis, CE is very efficient at separating DNA fragments for sequencing genome and applications related to forensic DNA typing examination. However, one disadvantage of CE is that very small sample volumes are required for loading [25]. This disadvantage hampers the application of CE to trace analysis of nucleotide due to poor limit of detection. An advantage of HPLC is that it is capable of analyzing classes of compounds with different polarities. HPLC also offers the option of derivatizing the analyte under investigation. Derivatization has tremendous advantage in improving selectivity of the separation and also in achieving much more rapid separations. A major disadvantage of HPLC is its lack of speed. An HPLC separation takes minutes to perform eliminating the possibility of a high throughput approach. A second disadvantage could be that much time is needed to re-equilibrate the stationary phase with a starting mobile phase composition. In addition, HPLC retention times are difficult to reproduce. As the stationary phase ages, retention times varies; they also vary from one column to the next.

One instrumental approach that could solve most of the problems outlined above is ambient pressure ion mobility spectrometry (IMS). IMS separates ions on the millisecond time scale and it has been shown to have good detection limits [26–27]. Another major advantage of IMS is that it provides absolute mobility values directly related to the structure of the compound. A number of publications have described the use of high-resolution electrospray ionization ion mobility spectrometry (ESI-IMS) [27–30] but ESI-IMS applications for ribonucleotide and ribonucleoside analysis appear to be absent. IMS coupled with a matrix assisted laser desorption ionization (MALDI) source and molecular mechanics/dynamics calculations has

also been used when investigating the gas phase conformation of trinucleotides and formation of zwitterions [31]. In addition to this study, MALDI-IMS combined with molecular modeling calculations was used to investigate the gas phase conformations and folding energetic of 16 deprotonated dinucleotides [32]. Collision-cross sections in this study were determined from arrival time distributions and the IMS was operated at low pressures and low-field strengths. This study was however useful in identifying three distinct families of dinucleotide conformers - stacked, H-bonded, and open.

The work reported in this short communication was designed to evaluate the potential for nucleotide and nucleoside analysis directly from liquid samples using ambient pressure ESI-IMS in negative ion mode. The negative ion mode was chosen for this study as we anticipated that most of the compounds can easily form negative ions. The drift times and reduced mobility of 16 nucleotides (12 ribonucleotide and 4 ribonucleoside) were reported for the first time. In addition, the detection limit and resolving power of each nucleotide was determined.

Experimental

Instrumentation

The ambient pressure ion mobility spectrometer used in this investigation was constructed at Washington State University, Pullman, WA. The instrument comprised of the following units: a) ESI source; b) reaction region; c) Bradbury-Nielsen ion gate; d) counter-flow ambient pressure drift region; e) aperture grid; f) faraday plate; and g) data acquisition system. Fig. 1 shows a schematic cross-sectional side view of the IMS.

The ESI solution was delivered with the use of a syringe pump obtained from KD Scientific (Holliston, MA, USA). The flow rate was set at $1 \mu\text{L min}^{-1}$ and delivered from a 500 μL syringe obtained from Hamilton (Reno, NV, USA). The syringe was connected to a 360 μm o.d. $\times$ 20 μm i.d. $\times$ 25 cm long fused silica capillary, Polymicro Technologies (Phoenix, AZ, USA) using a zero dead volume needle to capillary connector obtained from Upchurch Scientific (Oak Harbor, WA, USA). To ensure consistency in flow rate, the large tip aperture was connected to another fused silica capillary using a zero dead volume stainless steel union, Upchurch Scientific (Oak Harbor, WA). This second fused silica capillary (150 μm o.d. $\times$ 50 μm i.d. $\times$ 18 cm long) served as the emitter and was from Polymicro Technologies (Phoenix, AZ). The electrical contact was attached through the stainless steel zero dead volume union, Upchurch Scientific (Oak Harbor, WA) that connected the emitter with the fused silica capillary transfer line (360 μm o.d. $\times$ 75 μm i.d., $\sim$ 25 cm long).

Both the reaction and drift regions were constructed with stainless steel rings stacked in an interlocking design [30] for a total overall length of 23 cm. Each repeating unit in the reaction region, composed of a conductive stainless steel ring with a dimension of 60 mm (o.d.) $\times$ 50.4 mm (i.d.) $\times$ 2.5 mm (width) and an insulating ceramic ring with a dimension of 60 mm (o.d.) $\times$ 50.4 mm (i.d.) $\times$ 4.6 mm (width), stacked sequentially in an alumina tube. The drift region also composed of a conductive stainless steel ring with a dimension of 60 mm (o.d.) $\times$ 50.4 mm (i.d.) $\times$ 2.2 mm (width) and an insulating ceramic ring with a dimension of 60 mm (o.d.) $\times$ 50.4 mm (i.d.) $\times$ 1.6 mm (width), stacked sequentially in the alumina tube. To generate a uniform electric field down the tube, the stainless steel guard rings were connected to each other through 0.5 and 1 M Ω high-temperature resistors ($\pm$ 1%) obtained from Caddock Electronics Inc. (Thief River Falls, MN, USA) for the reaction and drift regions, respectively. The ceramic rings served to isolate the guard rings from the alumina tube as well as from each other.

A Bradbury-Nielsen gate ring divided the drift tube into a 5.6 cm long desolvation region and a 18.4 cm long drift region. A 9.5 kV voltage was normally applied to the first ring electrode

and the last ring electrode voltage was adjusted by variable resistor to be ~205 V referenced to ground. The drift voltage was dropped gradually across the drift tube via the resistor chain to form an electric field of 182 V cm^{-1} in the desolvation region and 487 V cm^{-1} in the drift region. The lower electric field in the desolvation region allowed solvated ions to spend more time in the heated drift gas to get more efficient desolvation prior to injection into the drift region. The Bradbury-Nielsen gate was made of electrically isolated alternating parallel Alloy 46 wires (76 μm in diameter) obtained from California Fine Wire Co. (Grover Beach, CA, USA), and spaced 0.25 mm apart [33,34]. The gate was “closed” by applying $\pm 50 \text{ V}$ to adjacent wires so that a $\sim 1550 \text{ V cm}^{-1}$ closing field was placed orthogonal to the drift tube of the IMS. As positive or negative ions approached the gate, they were trapped on the negative and positive wires, thereby preventing them from passing through the gate. When the gates were “open” all gate wires were pulsed to a single voltage appropriate to the gate’s position in the drift electric field.

Under the influence of the applied electric field, ions in the drift region were directed towards a stainless steel Faraday plate collector electrode against a counter-flow of atmospheric pressure drift gas at a fixed flow rate. The ratio of the drift velocity of a given ion to the applied field strength generated the ion mobility constant for the ion. An aperture grid, made in a similar manner as the Bradbury-Nielsen ion gate with the exception that adjacent wires were in common, was placed right in front of the terminal Faraday plate (60 mm in diameter) with $\sim 0.25 \text{ mm}$ spacing. The function of the aperture grid was to shield the incoming ion cloud from the detector prior to its arrival and reduce peak broadening. The counter flow drift gas was introduced to the drift tube near the tube terminus through a hollow drift ceramic ring which had 8 radially distributed apertures. The drift tube oven was constructed from two pieces of 12.5 cm long aluminum cylinder with 2 heating cartridges (Heatcon, Seattle, WA) embedded inside each cylinder. The electronics system used for the instrument included a high voltage power supply for the ESI emitter, a high voltage power supply for the drift tube, a two-channel temperature controller for the drift tube oven, a gate controller, and a current amplifier for signal amplification. Response of the Faraday detector was processed with a Model 427 current amplifier (Keithley Instruments, Cleveland, OH, USA) whose amplification was 10^9 V A^{-1} . The resulting signal was sent to a personal computer (operating Labview 7.0 code developed at WSU) through a SHC68-68-EPM noise rejecting shielded cable, National Instruments (Austin, TX, USA). The resulting text file data was then processed using Igor Pro 5.0.3, WaveMetrics (Portland, OR, USA).

Safety Considerations

Operation of the ESI source and IMS tube was conducted at high voltages, thus only trained personnel were allowed to operate the instrument. Warning signs were posted to warn about site clearance whenever the instrument was in operation mode.

Chemicals & Reagents

Ammonium acetate used in this study was purchased from Sigma Aldrich Chemical Company (St. Louis, MO, USA). The methanol was HPLC grade and was obtained from J.T. Baker (Phillipsburg, NJ, USA). Water used was 18 M Ω deionized, prepared by Barnstead Nanopure Water Systems. Adenosine, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), cytidine, cytidine monophosphate (CMP), cytidine diphosphate (CDP), cytidine triphosphate (CTP), guanosine, guanosine monophosphate, (GMP), guanosine diphosphate (GDP), uridine, uridine monophosphate (UMP), uridine diphosphate (UDP), and uridine triphosphate (UTP) were purchased from Sigma Aldrich Chemical Company. Standards solutions used in our analysis were prepared by dissolving each nucleotide samples from the manufacturer with the ESI solvent described below to achieve a concentration of 10 μM .

General Operating Conditions

The electrospray ionization was induced by applying a potential difference of ~3 kV between the electrospray emitter and the target focus screen (16 mesh stainless steel), which also served as the first ring electrode for the drift tube. 9.5 kV was applied to the first ring electrode and 12.5 kV was used for the electrospray emitter. It should be noted that the effective drift voltage across the drift region was the potential difference between the gate ring and the aperture grid ring (typically ~8.5 kV) but not total applied voltage. The drift tube was heated at 170–200 °C. N₂, used as counter flow drift gas throughout these experiments, was introduced to the drift tube at a constant flow rate of 1000 mL min⁻¹. Its purpose was to help evaporate the solvent from the electrospray produced charged droplets and to sweep out neutral interferences during spectral acquisitions. All experiments were carried out at atmospheric pressure which typically ranged from 680 to 705 Torr. The solvent used in the negative ion mode was a mixture of 90% MeOH/5% H₂O/5% NH₄Ac. The syringe pump was set at 1 µL min⁻¹ to deliver solution to the spray emitter. The charged droplets emitting from the ionization source were further desolvated by the heated drift gas while migrating slowly in the desolvation region. Desolvated ions were then injected into the drift region by opening the ion gate for a brief 150 µs pulse width at a frequency of 28 Hz. The current amplifier gain was usually set at 10⁹ V A⁻¹. Each IMS spectrum was data averaged from 100 gate pulses. Ten IMS spectra were acquired consecutively to obtain one averaged data point (peak intensity).

Calculations

In ambient pressure IMS measurements, the reduced mobility of an ion under normalized conditions of temperature and pressure is given as [35,36]:

$$K_0 = \left(\frac{L^2}{V \cdot t_d} \right) \left(\frac{273.15}{T} \right) \left(\frac{P}{760} \right) \quad (1)$$

where L (cm) is the length of an IMS drift cell, V is the voltage drop across L, T is the drift gas temperature (K) and P is the drift gas pressure (Torr) of the drift region where ion mobility, K₀ (cm² V⁻¹ s⁻¹), was determined. Under these standard temperature (273.15 K) and pressure (760 Torr) conditions the density of the drift gas molecules were normalized.

IMS resolving power was previously defined as the drift time (t_d) of the ion divided by the temporal width-at-half height (w_{0.5}) of the mobility peak [37–39]. This relationship is given by eq 2.

$$R_m = \frac{t_d}{w_{0.5}} \quad (2)$$

where R_m is the measured resolving power. The peak width at half height is assumed to be the final peak width accounting for all band broadening processes in the IMS system. Detection limits for all nucleotides were determined from the spectra [34] and all measurements were carried out close to the detection limit values. Thus, a linear response was assumed to estimate the detection limits as follows:

$$R_{(t)} = S[C] + B \quad (3)$$

where R_(t) is the response (nanoampere, nA), S is sensitivity (nA ppm_v⁻¹), [C] is the concentration (µM) and B is the background current (nA). Given that the response of an

unknown A ($R_{(t)A}$) is $R_{(t)A}-B$ and $R_{(t)A}-B = S[C]$ (Eq. 3), then at the detection limit, where $R_{(t)A} = 3\sigma$ (σ is the root mean square of the noise above the background current), it follows that:

$$R_{(t)A} = S[C] = 3\sigma \quad (4)$$

Rearranging Eq. (4) leads to Eq. (5):

$$[C]_D = \frac{3\sigma}{S} \quad (5)$$

where $[C]_D$ is the minimum detectable concentration, and $S = R_{(t)}/C$ and C is the concentration at which detection limit was determined.

Results & Discussion

Reduced mobility

Ribonucleotide and ribonucleoside standard solutions were introduced into the IMS to determine their reduced mobility in nitrogen. To ensure that the reduced mobility reported in this work were accurate and to prevent day-to-day variations in temperature and pressure, a calibration standard was also used to calculate reduced mobilities of the nucleotides. This investigation used 2,4,6-trinitrotoluene (TNT) as the calibration standard in the negative ion mode. TNT was chosen as the calibration standard since the reduced mobility has been reported extensively in the literature. The reduced mobility of TNT was reported as $1.54 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. This value matched that reported in the literature for TNT ($1.54 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$) in nitrogen drift gas [40].

The K_0 values for all 12 ribonucleotides and 4 ribonucleoside studied in this investigation were calculated from drift times obtained after sample introduction and are listed in Table 1. Three replicate measurements were taken for all nucleotides and in all cases drift times were found to be similar. Fig. 2(a) shows the spectra obtained for adenosine, AMP, ADP, and ATP. Three peaks were identified for adenosine. These peaks had K_0 values of 1.85 ± 0.02 , 1.21 ± 0.01 , and $1.15 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, respectively. Two peaks were identified for AMP and two peaks were identified for ADP with reduced mobilities at 1.86 ± 0.02 and $1.15 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ for AMP, and 1.86 ± 0.02 and $1.05 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ for ADP. Only one peak was identified for ATP with a K_0 value of $1.00 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$.

Fig. 2(b) shows spectra obtained for cytidine, CMP, CDP, and CTP. Two peaks for each compound were produced for cytidine, CDP, and CTP. The K_0 values for cytidine were observed at 1.38 ± 0.01 and $1.28 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The K_0 value for CMP occurred at $1.22 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The K_0 values for CDP were 1.22 ± 0.01 and $1.12 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, and the K_0 values for CTP were 1.12 ± 0.02 and $1.05 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$.

Fig. 2(c) displays the spectra obtained for guanosine, GMP, GDP, and GTP. The K_0 value for guanosine was determined to be $1.27 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The K_0 value for GMP was $1.18 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The K_0 values for GDP were 1.18 ± 0.02 and $1.07 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, and the K_0 values for GTP were 1.07 ± 0.02 and $0.99 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$.

Fig. 2(d) depicts the spectra obtained for uridine, UMP, UDP, and UTP. Three peaks were identified for uridine with K_0 values of 2.02 ± 0.02 , 1.37 ± 0.01 , and $1.30 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. The K_0 values of CMP and CDP were 1.23 ± 0.01 , and $1.13 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, respectively, and those of CTP were 1.75 ± 0.01 , 1.13 ± 0.01 , and $1.05 \pm 0.01 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$.

Variation in the electric field, temperature, and pressure of the drift region could result in inaccurate measurements of K_0 values. To correct for variations in these instrumental parameters, standards were used to calibrate the instruments. A 2% difference in K_0 value was considered acceptable. The fact that the K_0 value reported for the daily calibration standard (TNT), used in this investigation compared well with literature values, demonstrated that the K_0 values reported for the respective nucleotides were acceptable. Because this is the first time that ion mobility has been reported for electrospray ionization of nucleotides, no literature K_0 values for these compounds are available. Nucleotides have been reported by MALDI low pressure ion mobility but reduced mobility values were not reported in those studies [31–32].

Limit of detection and resolving power

Detection limits were calculated for the nucleotides and nucleosides studied using eq 4. The minimum limit of detection of 15 picomoles (pmol) was calculated for adenosine whereas the maximum limit of detection (LOD) of 300 pmol was calculated for UTP. Table 1 lists all detection limits for the 12 ribonucleotides and 4 ribonucleosides studied. The results demonstrated that IMS can be used to detect nucleotides and nucleosides in the low picomoles range.

Resolving power for the 16 nucleotides are also listed in Table 1. The maximum resolving power of 56 was reported for ATP and the minimum value of 41 was reported for guanosine. Fig. 3 shows responses to mixtures of the nucleotide sets studied. Mixture 1, spectra labeled (a) in Fig. 3, was composed of adenosine, AMP, ADP, and ATP. Mixture 2, spectra labeled (b) in Fig. 3, was composed of cytidine, CMP, CDP, and CTP. Mixture 3, spectra labeled (c) in Fig. 3, was composed of guanosine, GMP, GDP, and GTP. Mixture 4, spectra labeled (d) in Fig. 3, was composed of uridine, UMP, UDP, and UTP. The reduced mobilities of the respective mixtures matched those obtained for the single component ribonucleotides and ribonucleosides. In some cases baseline resolution was obtained. The IMS used in this investigation was not considered a high-resolving power instrument. However, with a resolving power in the range of 41–56, it was possible to resolve most of the response ions buried in the mixtures. Thorough analysis of the mixture, data showed that an IMS constructed with a resolving power in the range of 80–90 will be sufficient to resolve all 16 nucleotides. One of the benefits of ambient pressure IMS is that it offers high resolving power. An IMS with a resolving power greater than 90 can be constructed by increasing the length of the drift tube, lowering the temperature, and raising the drift gas pressure. Recent data has also shown that optimization of the drift tube voltage and initial gate pulse width could result in improved resolving power [41]. These possibilities suggest that IMS could be conveniently used for rapid separation of nucleotides and nucleosides under ambient conditions and it appeared conceivable that further optimizations may lead to increased values of resolving power. Improvement in resolving power for IMS systems coupled with the speed of analysis should therefore hold great promise for nucleotide and nucleoside analysis in the area of separating DNA fragments in genome sequencing and also for forensics DNA typing examinations used for the identification of blood stains in crime scenes and paternity testing.

Summary and Conclusion

An ambient pressure IMS with resolving power in the range of 41–56 was evaluated for detecting and separating ribonucleotides and ribonucleosides. Reduced mobilities for the 12 ribonucleotides and 4 ribonucleosides investigated were reproducible within 1–3%. Detection limits reported range from 15–300 pmols. The preliminary results suggested that constructing a high-resolving power IMS with resolving power of at least 80 could provide rapid and sensitive detection and separation of ribonucleotides and ribonucleosides. It is anticipated that with improved IMS resolving power, any ribonucleotide and deoxyribonucleotide mixture may

be resolved by IMS analysis. Furthermore, future nucleotide and nucleoside analysis would particularly benefit from the speed of IMS instrumentation which could serve as an attractive tool for high-throughput analysis. This short report demonstrates that it was possible to use IMS for the separation of nucleotides and nucleosides in less than one second. The application holds great promise for nucleotide and nucleoside analysis in the area of separating DNA fragments in genome sequencing and also for forensics DNA typing examinations used for the identification of blood stains in crime scenes and paternity testing. Furthermore, with ambient pressure IMS speed of analysis, good detection limit, absolute mobility values, this technology when successfully integrated with mass spectrometry and molecular modeling could well be a suitable replacement for CE and LC after PCR in sequencing genome.

Acknowledgments

This work was partially supported by the EPA (Grant Nos. X-97031101-0 and X-97031102-0). The authors are mostly thankful to Dr. Molly M. Gribb for her support in this work.

References

1. Li D-F, Li J, Ma L, Zhang L, Lu Y-T. FEBS Letters 2006;580:4325. [PubMed: 16842786]
2. Zhou J, Low PS. J Biol Chem 2001;276:38147. [PubMed: 11477080]
3. Cok SJ, Acton SJ, Sexton AE, Morrison AR. J Biol Chem 2004;279:8196. [PubMed: 14662769]
4. Koomen JN, Russell WK, Tichy SE, Russell DH. J Mass Spectrom 2002;37:357. [PubMed: 11948842]
5. Hofstadler SA, Griffey RH. Chem Rev 2001;101:377. [PubMed: 11712252]
6. Guo BC. Anal Chem 1991;71:333R.
7. McCord B, Hartzell-Baguley B, King S. Methods in Mol Biol 2008;384:415. [PubMed: 18392577]
8. Xu G, Shi X, Zhao C, Yuan K, Weng Q, Gao P, Tian J. Methods in Mol Biol 2008;384:441. [PubMed: 18392579]
9. Fundador EV, Choudhary D, Schenkman JB, Rusling JF. Anal Chem 2008;80:2212. [PubMed: 18266391]
10. Yu S, Geng J, Zhou P, Wang J, Feng A, Chen X, Tong H, Hu J. Anal Chim Acta 2008;611:173. [PubMed: 18328318]
11. Aquadro CF, Greenberg BD. Genetics 1983;103:287. [PubMed: 6299878]
12. Warnke U, Rappell C, Meier H, Kloft C, Galanski M, Hartinger CG, Keppler BK, Jaehde U. ChemBioChem 2004;5:1523. [PubMed: 15515086]
13. Cunnliffe JN, Sunahara RK, Kennedy RT. Anal Chem 2006;78:1731. [PubMed: 16536404]
14. Gong M, Nikcervic I, Wehmeyer KR, Limbach PA, Heineman WR. Electrophoresis 2008;29:1415. [PubMed: 18324729]
15. Kenefic LJ, Beaudry J, Trim C, Huynh L, Zanecki S, Mathews M, Schupp J, Van Ert M, Keim P. Journal of Microbiological methods 2008;73:269. [PubMed: 18237793]
16. Lin S-W, Chang G-L, Lin C-H. J Chromatogr A 2008;1192:198. [PubMed: 18407282]
17. Sotgia S, Carru C, Franconi F, Fiori PB, Manca S, Pettinato S, Magliona S, Ginanneschi R, Deiana L, Zinellu A. J Chromatogr A 2008;1185:145. [PubMed: 18255082]
18. Liu CC, Huang JS, Tyrrell DLJ, Dovichi NJ. Electrophoresis 2005;26:1424. [PubMed: 15761917]
19. Sar DG, Montes-Bayón M, González EB, Sanz-Medel A. J Anal At Spectrom 2006;21:861.
20. Pruvost A, Théodoro F, Agrofoglio L, Negredo E, Bénech H. J Mass Spectrom 2008;43:224. [PubMed: 17935070]
21. Saux TL, Chhun S, Rey E, Launay O, Weiss L, Viard J-P, Pons G, Jullien V. J Chromatogr B 2008;865:81.
22. Klawitter J, Schmitz V, Klawitter J, Leibfritz D, Christians U. Anal Biochem 2007;365:230. [PubMed: 17475198]
23. St Claire RL III. Rapid Commun Mass Spectrom 2000;14:1625. [PubMed: 10960917]

24. Soga T, Ishikawa T, Igarashi S, Sugawara K, Kakazu Y, Tomita M. *J Chromatogr A* 2007;1159:125. [PubMed: 17543971]
25. Lai, CC.; Kelly, JA. Abstract of papers, 224th ACS National Meeting; Boston, MA, USA. August 18–22 2002;
26. Kanu AB, Wu C, Hill HH Jr. *Anal Chim Acta* 2008;610:125. [PubMed: 18267149]
27. Asbury GR, Hill HH Jr. *J Chromatogr A* 2000;902:433. [PubMed: 11192176]
28. Dugourd P, Hudgins RR, Clemmer DE, Jarrold MF. *Rev Sci Instrum* 1997;119:1122.
29. Srebalus CA, Li J, Marshall WS, Clemmer DE. *Anal Chem* 1999;71:3918. [PubMed: 10500479]
30. Wu C, Siems WF, Asbury GR, Hill HH Jr. *Anal Chem* 1998;70:4929.
31. Gidden J, Bowers MT. *J Am Soc Mass Spectrom* 2003;14:161. [PubMed: 12586465]
32. Gidden J, Bowers MT. *Eur Phys J D* 2002;20:409.
33. Eatherton, RL. PhD Thesis. Washington State University; 1987. Development in Ion Mobility Detection for Capillary Chromatography.
34. Kanu AB, Hill HH Jr, Gribb MM, Walters RN. *J Environ Monit* 2007;9:51. [PubMed: 17213942]
35. Carr, TW. *Plasma Chromatography*. Plenum Publication Corporation; New York: 1984.
36. Spangler GE, Vora KN, Carrico JP. *J Phys E Sci Instrum* 1986;19:191.
37. Spangler GE. *Int J Mass Spectrom* 2002;220:399.
38. Siems WF, Wu C, Tarver EE, Hill HH, Larsen PR, McMinn DG. *Anal Chem* 1994;66:4195.
39. Rokushika S, Hatano H, Baim MA, Hill HH Jr. *Anal Chem* 1985;57:1902.
40. Shumate C, St Louis RH, Hill HH Jr. *J Chromatogr* 1986;373:141. [PubMed: 3543046]
41. Kanu AB, Gribb MM, Hill HH Jr. *Anal Chem* 2008;80:6610. [PubMed: 18683951]

Figure 1.
Schematic cross-sectional side view of an ambient pressure ion mobility spectrometer.

Figure 2. ESI-IMS spectra for (a) adenosine, AMP, ADP, and ATP (b) cytidine, CMP, CDP and CTP (c) guanosine, GMP, GDP and GTP (d) uridine, UMP, UDP and UTP obtained in the negative ion mode. The reduced mobility values are indicated on the plots.

Figure 3. ESI-IMS spectra for nucleotide mixtures. Spectrum (a) was a mixture of adenosine, AMP, ADP, and ATP. Spectrum (b) was a mixture of cytidine, CMP, CDP, and CTP. Spectrum (c) was a mixture of guanosine, GMP, GDP, and GTP whereas spectrum (d) was a mixture of uridine, UMP, UDP, and UTP. The numbers indicated on the plot were the reduced mobilities of the different ions observed.

Table 1

Reduced mobility values, resolving power, and limits of detection (LOD) for nucleotides using electrospray ionization IMS.

Compound (CAS)	MWt/g mol ⁻¹	K ₀ (cm ² V ⁻¹ s ⁻¹)	Resolving power	LOD/picomoles
Adenosine (58-61-7)	267	1.85, 1.21, 1.15	48	15 ± 3
AMP (84-21-9)	347	1.86, 1.15	50	85 ± 9
ADP (58-64-0)	427	1.86, 1.05	55	35 ± 6
ATP ((56-65-5)	507	1.00	56	52 ± 8
Cytidine (65-46-3)	243	1.38, 1.28	42	78 ± 9
CMP (63-37-6)	323	1.22	47	90 ± 12
CDP (34393-59-4)	403	1.22, 1.12	51	110 ± 7
CTP (65-47-4)	483	1.12, 1.05	55	210 ± 25
Guanosine (118-00-3)	283	1.27	41	85 ± 8
GMP (85-32-5)	363	1.18	43	82 ± 4
GDP (146-91-8)	443	1.17, 1.07	48	92 ± 12
GTP (56001-37-7)	523	1.07, 0.99	50	105 ± 9
Uridine (58-96-8)	244	2.02, 1.37, 1.30	43	25 ± 5
UMP (58-97-9)	324	1.23	47	32 ± 4
UDP (58-98-0)	404	1.13	51	225 ± 13
UTP (63-39-8)	484	1.75, 1.13, 1.05	53	300 ± 15