The Use of Shift Reagents in Ion Mobility-Mass Spectrometry: Studies on the Complexation of an Active Pharmaceutical Ingredient with Polyethylene Glycol Excipients

Mark D. Howdle,[a] Christine Eckers,[b] Alice M.-F. Laures,[b] and Colin S. Creaser[a]

[a] Centre for Analytical Science, Department of Chemistry, Loughborough University, Leicestershire, United Kingdom
[b] GlaxoSmithKline, Stevenage, United Kingdom

Gas-phase ion mobility studies of mixtures containing polyethylene glycols (PEG) and an active pharmaceutical ingredient (API), lamivudine, have been carried out using electrospray ionization-ion mobility spectrometry-quadrupole-time-of-flight mass spectrometry (ESI-IMS-Q-TOF). In addition to protonated and cationized PEG oligomers, a series of high molecular weight ions were observed and identified as noncovalent complexes formed between lamivudine and PEG oligomers. The noncovalent complex ions were dissociated using collision induced dissociation (CID) after separation in the ion mobility drift tube to recover the protonated lamivudine free from interfering matrix ions and with a drift time associated with the precursor complex. The potential of PEG excipients to act as "shift reagents," which enhance selectivity by moving the mass/mobility locus to an area of the spectrum away from interferences, is demonstrated for the analysis of lamivudine in a Combivir formulation containing PEG and lamivudine. (J Am Soc Mass Spectrom 2009, 20, 1–9) © 2009 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry

Ion mobility spectrometry (IMS) [1] is a gas-phase electrophoretic technique that provides rapid separations of gas-phase ions on the milliseconds timescale. The theory and applications of IMS have been presented in a number of reviews [2–5]. Gas-phase ions introduced into the IMS spectrometer are accelerated through a drift tube, under the influence of a weak electric field gradient and in the presence of a neutral buffer gas (typically nitrogen, helium, or air), resulting in the separation of ions on the basis of differing mobilities. Ion mobility, (K (cm^2 V^{-1} s^{-1}), can be determined from the time taken for an ion to traverse the drift tube,

$$K = \frac{V_d}{E} \equiv \frac{l^2}{t_d v} \quad (1)$$

where E (V cm^{-1}) is the electric field experienced by the ion, v (V) is the total applied voltage used to create the electric field gradient in the drift cell, V_d (cm s^{-1}) is the average drift velocity of the ion, t_d(s) is the ion drift time, and l (cm) is the length of the drift tube. The mobility of a gas-phase ion is related to its collision cross section (i.e., size and shape) [6],

$$K = \left(\frac{3ze}{16N}\right)\left(\frac{2\pi}{\mu k_B T}\right)^{1/2}\left(\frac{1}{\Omega_D}\right) \quad (2)$$

where z is the numerical charge, e is the charge on the ion, N is the number density of the buffer gas, μ is the reduced mass of the ion and buffer gas, k_B is the Boltzmann constant, T is the temperature, and Ω_D is the collision cross-section.

IMS has been reported for the analysis of active pharmaceutical ingredients [7–10], veterinary drugs [11], pesticides [12], and narcotics [13–16], using atmospheric or low-pressure [<10 Torr (1333 Pa)] drift tubes. Hyphenated instruments combining low field ion mobility separations with MS analyzers (IM-MS) [17] have been used to identify pharmaceuticals [18–20] and narcotics [21–23]. Desorption electrospray-IM-MS has been reported for the direct analysis of active pharmaceutical ingredients in pharmaceutical formulations containing PEG [18], demonstrating the benefits offered by ion mobility separations for pharmaceutical formulation analyses.

Address reprint requests to Prof. C. S. Creaser, Centre for Analytical Science, Department of Chemistry, Loughborough University, Leicestershire LE11 3TU, UK. E-mail: c.s.creaser@lboro.ac.uk

© 2009 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry.
1044-0305/09/$32.00
doi:10.1016/j.jasms.2008.10.002

Published online October 9, 2008
Received August 20, 2008
Revised September 30, 2008
Accepted October 1, 2008

Currently, IMS separations offer low full width at half height (FWHH) resolutions with typical values ranging from 10 to 40, limiting the applications of this technique for the analysis of ions with the same charge and similar collision cross sections. Increasing the electric field strength results in relatively higher resolving power (≈200) [24]. However, a consequence of high electric field strength is that the drift gas pressure must be increased proportionally to maintain a low E/N ratio ($<2 \times 10^{-17}$ V cm^{-2}), required for field-independent mobilities, which may result in lower ion transmission. The temperature of the drift tube system may also be decreased to improve resolution, although this favors cluster formation, making the identification of unknown samples difficult [25]. The use of drift gasses other than nitrogen, helium, and air has also been used to enhance IMS separations [26, 27]. Hill and Asbury demonstrated that the selectivity of an IMS separation may be altered by employing drift gasses of different polarizability [28].

An alternative approach to enhancing selectivity without altering the instrumental configurations is to use a shift reagent. A shift reagent is defined as a species that reacts or complexes with an analyte to form a gas-phase ion of lower mobility, which increases (shifts) drift time and enhances separation. Shift reagents were first proposed by Creaser et al., on the basis of studies of the complexation of protonated amines with crown ethers, using a tandem quadrupole ion trap/ion mobility spectrometer [29, 30]. These studies demonstrated enhanced ion mobility separations for the complexes compared to the free protonated amines. Reports of IMS studies of noncovalent complexes incorporating polyethers have included the analysis of amino acids [31], amines [29, 30, 32], and peptides [33, 34] with crown ether and linear polyether reagents. IMS separations of carbohydrates complexed to metal cations demonstrated the tunable selectivity enhancements possible by altering the metal salt [35, 36], and recently functionalized lanthanides have also been applied as shift reagents to provide selective IMS separations [37].

PEG oligomers are widely used in pharmaceutical formulations as dosing vehicles, and the collisional cross sections of sodiated PEG oligomers have been measured using matrix assisted laser desorption ionization (MALDI) [38] over the temperature range 80–600 K [39–41]. This work was extended to the lithium and caesium cationized adducts with PEG and a synthetic derivative [42]. High-field asymmetric waveform ion mobility spectrometry (FAIMS) [43, 44] and differential mobility spectrometry (DMS) [45] have also been used to characterize mixtures of PEG oligomers.

In this paper, we describe an IM-MS study of the gas-phase, noncovalent complexes of lamivudine, an active pharmaceutical ingredient, with PEG oligomers, and discuss the potential of PEGs as shift reagents to simplify and improve the selectivity for IM-MS analyses of formulated pharmaceutical ingredients.

Experimental

Materials

HPLC grade methanol and analytical reagent (AR) grade glacial acetic acid were purchased from Fisher Scientific (Loughborough, UK). Mass spectroscopy grade (Puriss, p.a.) formic acid and polyethylene glycol (average molecular weight 400) were purchased from Sigma-Aldrich (Gillingham, UK). Distilled and deionized water was obtained in-house using a Triple Red water purification system (Triple Red, Long Crendon, UK). Lamivudine and Combivir tablets were obtained from GlaxoSmithKline (Stevenage, UK).

Sample Preparation

Solutions of 49.5/49.5/1 (vol/vol/vol) methanol/water/formic acid were used in all experiments. Stock solutions of lamivudine (1 mg mL^{-1}) were prepared in methanol and solutions of polyethylene glycol (2 mg mL^{-1}) were prepared in distilled and deionized water. A stock solution of Combivir was prepared by grinding a Combivir tablet and dissolving in 100 mL of 49.5/49.5/1 (vol/vol/vol) methanol/water/formic acid. Aliquots of the Combivir stock solution were centrifuged (Eppendorf, Hamburg, Germany) at 13,200 rpm for 3.5 min to ensure that insoluble matter was not transferred to the mass spectrometer.

Ion Mobility-Mass Spectrometry Analysis

IM-MS experiments on lamivudine and PEG mixtures were carried out using a prototype low-pressure ion mobility-quadrupole-time-of-flight mass spectrometer (IMS-Q-TOF) based upon a Q-TOF Ultima spectrometer (Waters, Manchester, UK), which has been described in detail elsewhere [18]. Sample solutions were delivered from a gastight glass syringe (500 μL; SGE, Sydney, Australia) to the ESI probe by an integrated syringe pump (5 μL min^{-1}). Electrosprayed ion populations were trapped radially using an RF only trap and periodically gated into the mobility device [15 ms repetition period, 200 μs pulse width, concurrent flow of nitrogen gas at ≈2 mbar (200 Pa)]. A voltage gradient (13.16 V cm^{-1}) was applied to the linear ion mobility drift tube (15.2 cm long). The instrument was operated in positive ion mode with the ESI capillary set to 3.5 kV. The nitrogen desolvation gas was set to 500 L h^{-1}, the source temperature to 120 °C, the desolvation temperature to 180 °C, and the cone voltage to 35 V. The quadrupole mass filter was operated in wide-band-pass mode (m/z 50 to 1300) and the RF hexapole collision cell was operated without collision gas (10 V collision cell energy) to transmit ions into the orthogonal acceleration TOF region. Single ion mobility spectra were acquired by measuring 200 sequential TOF scans, where each TOF scan spaced 65 μs apart corresponds to a "bin number", over a period of 15 ms from the point of ion

injection into the drift cell. IM-MS data were accumulated for a 5 s scan time and processed automatically by combining the mass spectra for all ion mobility separations. Acquired data were presented as a plot of time (TOF bin number) against ion intensity (total ion mobility response or selected ion mobility response).

For tandem mass spectrometric experiments, CID was used; the precursor ion exiting the drift cell region was selected using the quadrupole mass filter and accelerated through the collision cell [0.5 mbar (50 Pa) argon, corresponding cell pressure of ≈0.7 Pa, 20–50 V collision energy]. Product ion data were obtained by combining one hundred 5 s accumulated scans using Driftscope software (Waters, Manchester, UK).

The analysis of Combivir tablets was carried out using a Synapt IM-MS spectrometer [46] (Waters, Manchester, UK) operated in positive ion mode with the ESI capillary set to 3.0 kV. The nitrogen desolvation gas was set to 800 L h^{-1}, the source temperature to 100 °C, the desolvation temperature to 150 °C, the cone voltage to 40 V and the nitrogen cone gas flow to 50 L h^{-1}. The quadrupole mass filter was operated in wide-band-pass mode (analyzing *m/z* 100–1000). The nitrogen IMS gas flow was set to 20 mL min^{-1} and the IMS traveling wave velocity to 300 m s^{-1} with a wave height of 9.0 V. Single ion mobility spectra were acquired by measuring 200 sequential TOF pushes (45 μs). IM-MS data were accumulated for a 2 s scan time and processed automatically by combining the mass spectra for all ion mobility separations. Sample solutions were delivered from a gastight glass syringe (250 μL; SGE) to the ESI probe by an integrated syringe pump (2 μL min^{-1}). For tandem mass spectrometric experiments the precursor ions were selected using the quadrupole mass filter, before acceleration into the trapping region (5 mL min^{-1} argon corresponding to a trap pressure of 2.98×10^{-2} mbar (3.97 Pa), set to 25 V) where they were subjected to partial fragmentation before entering the drift cell. Ions were mobility separated in the drift cell before undergoing further partial fragmentation in the transfer region (5 mL min^{-1} argon corresponding to a trap pressure of 2.98×10^{-2} mbar (3.97 Pa), set to 6 V) and detected in the TOF region, providing drift time measurements and tandem mass spectra for the precursor ion and fragments created in the trapping region.

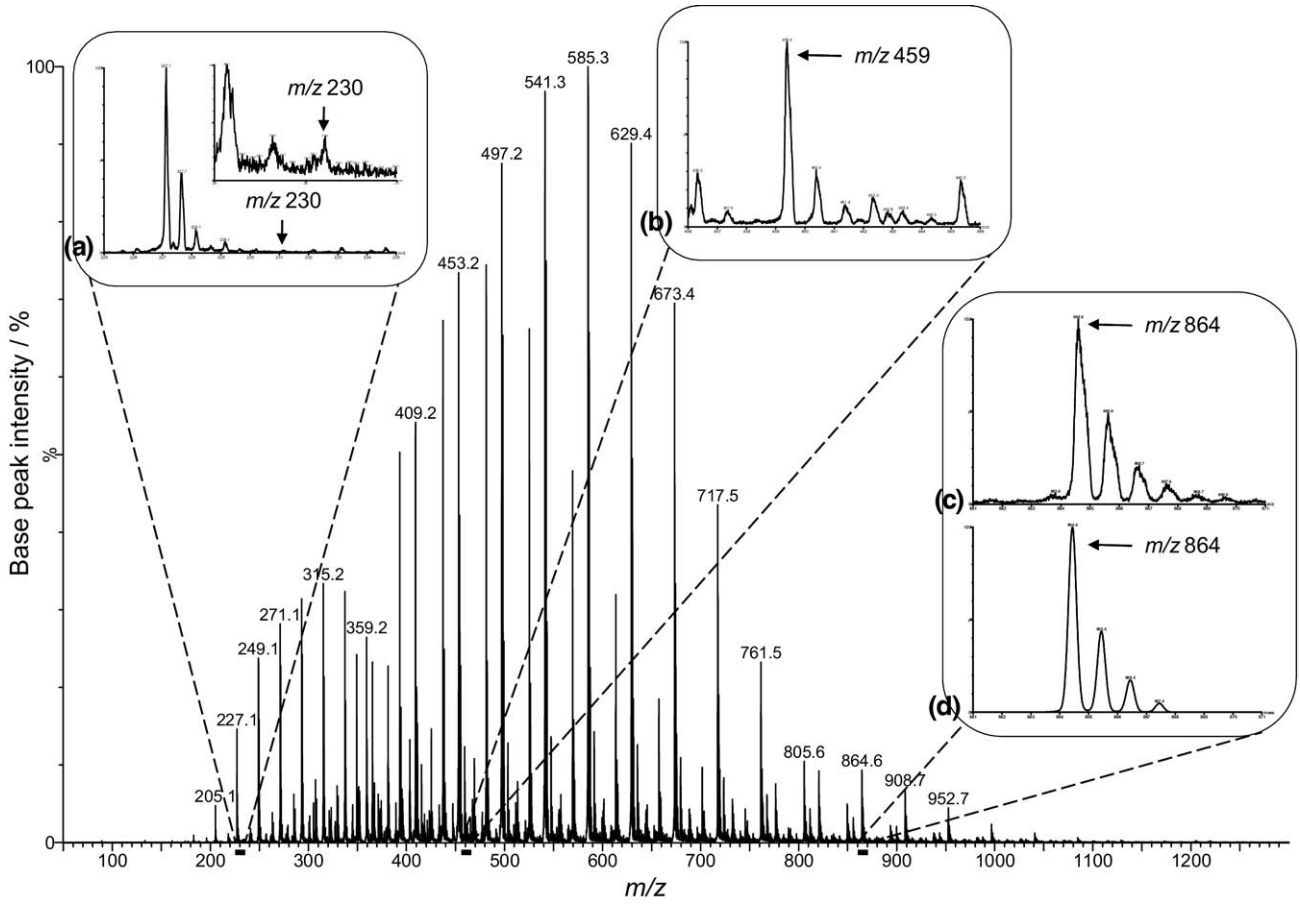

Figure 1. Mass spectrum of a mixture of lamivudine (10 μg mL^{-1}) and PEG 400 (1000 μg mL^{-1}) without ion mobility separation, (**a**) expanded region around the protonated lamivudine ion (*m/z* 230), (**b**) expanded region around protonated lamivudine dimer and protonated PEG (n = 10) oligomer (*m/z* 459), (**c**) expanded region around the lamivudine/PEG complex ion (*m/z* 864, n = 14) and, (**d**) the predicted isotopic pattern of the lamivudine/PEG complex ion.

Results and Discussion

Analysis of Lamivudine/PEG Mixture

Infused solutions of lamivudine, an active pharmaceutical ingredient in formulations of Combivir, used to treat human immunodeficiency virus (HIV) infection, PEG 400 and lamivudine/PEG mixtures were analyzed using ESI-IM-MS, generating mass to charge and mobility data for the electrosprayed ions. The mass spectrum of a mixture containing lamivudine (10 μg mL^{-1}) and PEG 400 (1000 μg mL^{-1}) with concentrations selected to reflect those found in formulated pharmaceutical products is shown in Figure 1. The spectrum of the lamivudine/PEG mixture is very complex as a result of overlapping series of protonated and cationized PEG adduct ions. The *m/z* ratios of the PEG related

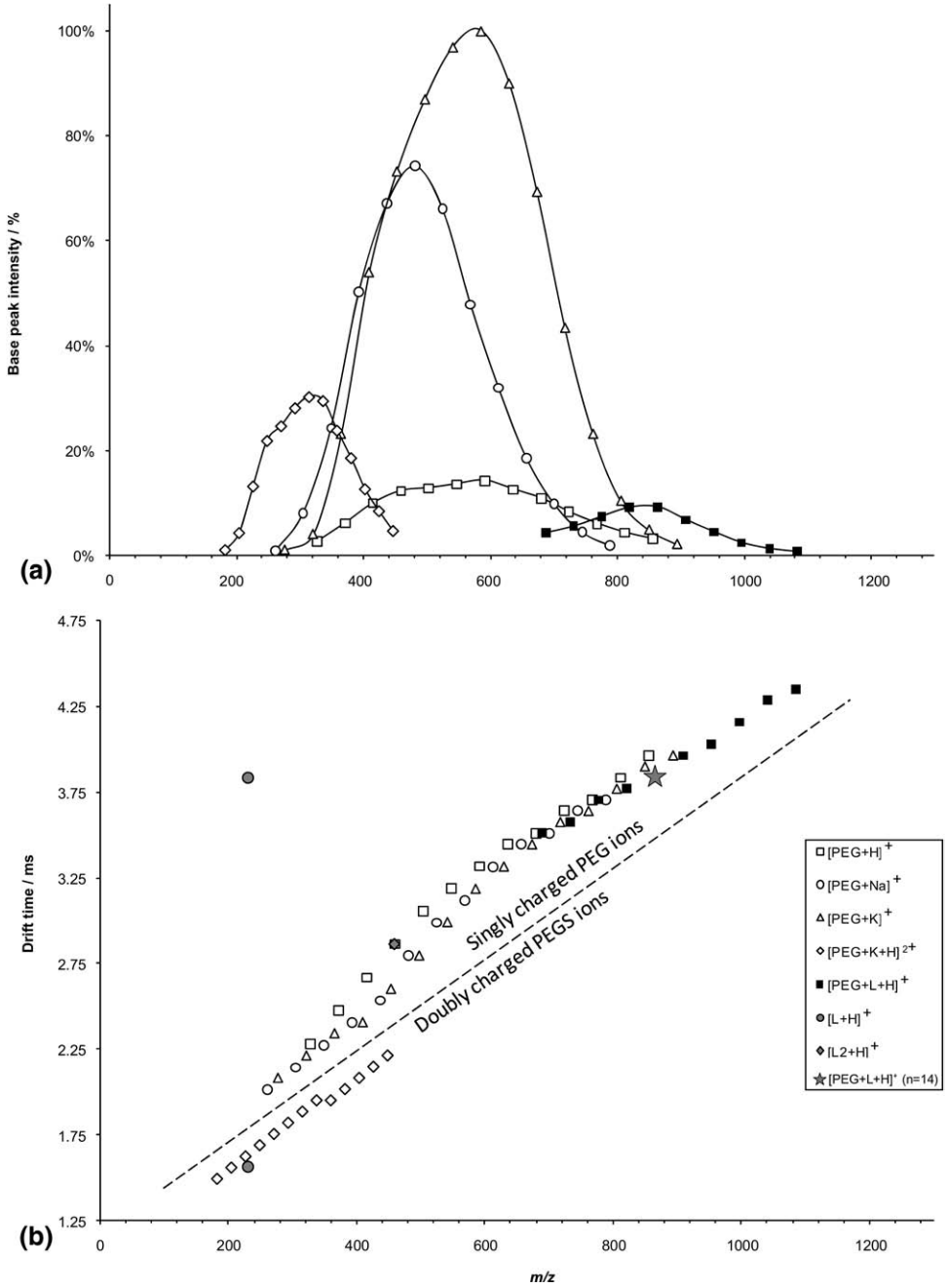

Figure 2. Ion series present in a mixture containing lamivudine (10 μg mL^{-1}) and PEG 400 (1000 μg mL^{-1}); (**a**) mass-to-charge/intensity plot displaying the most intense series of PEG ions and the lamivudine/PEG complex ion series and (**b**) mass-to-charge/ion mobility plot displaying the most intense series of PEG ions and the lamivudine/PEG complex ion series. The free protonated lamivudine and the protonated lamivudine after complexation with PEG are indicated by (filled circle) and (filled star), respectively.

signals are plotted against normalized intensity (%I) and ion mobility drift time (t_d) in Figure 2. In addition to the protonated PEG ion series, other series with 44 and 22 Th repeating units were observed at higher mass to charge ratios in the mass spectrum. These series are assigned as cationized (Na^+ and K^+) adducts of PEG. Drift time (t_d) increases with PEG oligomer chain length for the protonated and cationized ions, reflecting the increase in collision cross section with chain length [41]. The ion drift times (t_d)of the $[PEG + H]^+$, $[PEG + K]^+$, and $[PEG + Na]^+$oligomers were very similar, ±0.065 ms (equivalent to ±1 bin), with the exception of $n = 9$ where $[PEG + H]^+$ is separated by 0.13 ms (2 bins) from $[PEG + Na]^+$. The gas-phase ion mobilities of the $[PEG + Na]^+$ ions are all greater than, or equal, to the mobilities of the $[PEG + H]^+$ complexes. This is consistent with the mobilities of alkali metal/crown ether (18-crown-6) complexes reported by Bowers et al. [47], which were observed to decrease in the order of: $Li^+ > Na^+ > K^+ > Cs^+$. The proposed explanation [47] for the order of ion mobilities was that the crown ether forms a more tightly bound complex with ions of high charge density, such as lithium, to produce smaller collision cross sectional areas, whereas ions of greater radius and lower charge density have lower ion mobilities. The doubly charged ions, $[PEG + K + H]^{2+}$, have higher mobilities than the corresponding singly charged ions because mobility is directly proportional to charge state (eq 2), and this separation between singly and doubly charged PEG related ions is apparent in Figure 2. The separation of analytes by charge state has also been observed in proteomic studies using IM-MS [48]. Mobility separations based on charge provide reductions in spectral complexity at a given *m/z* range and signal to noise improvements, which have been demonstrated in human drug metabolism and pharmacokinetic (DMPK) studies using IM-MS [49].

Electrosprayed solutions of lamivudine (L) in the absence of PEG show $[L + H]^+$ (*m/z* 230) and $[L_2 + H]^+$ (*m/z* 459) ions (data not shown). However, in mixtures containing PEG, electrosprayed PEG molecules ionize efficiently and produce a large number of intense responses, which may mask the weaker signals from ions such as active pharmaceutical ingredients and related impurities, presenting difficulties for mass spectra interpretation [19]. Electrosprayed solutions of the simulated pharmaceutical formulation (Figure 1) display a weak response for protonated lamivudine $[L + H]^+$ at *m/z* 230 (Figure 1a), which is barely visible above the baseline. The signal intensity at *m/z* 459 is strong, but corresponds to an overlap of the lamivudine $[L_2 + H]^+$ dimer and a PEG oligomer ion $[PEG + H]^+$ ($n = 10$) that has the same nominal mass to charge ratio as the lamivudine dimer ion (Figure 1b).

In addition to the protonated and cationized PEG ion series, a series with a 44 Th repeating unit was observed at higher *m/z* and higher drift time than the protonated and cationized PEG oligomers (Figure 2) in the electrosprayed lamivudine/PEG mixture. These ions were assigned to a series of noncovalent complexes containing lamivudine and PEG oligomers of increasing chain length. For example, the ion at *m/z* 864 (Figure 1c) is assigned as a lamivudine/PEG, $[L + PEG + H]^+$ complex ($n = 14$; $C_{36}H_{70}N_3O_{18}S$). The theoretical isotopic pattern presented in Figure 1d compares well with the isotopic pattern observed experimentally in Figure 1c. A range of PEG oligomer chain lengths ($n = 10$–19) were observed in the $[L + PEG + H]^+$ series using IM-MS (Figure 2) with selected ions identified using tandem mass spectrometry.

Total ion and selected ion mobility responses for the lamivudine/PEG mixture are displayed in Figure 3. Selected ion responses for protonated lamivudine (*m/z* 230; Figure 3b), the lamivudine dimer and protonated PEG ions at *m/z* 459; ($n = 10$; Figure 3c), the protonated PEG ions at *m/z* 503 ($n = 11$; Figure 3d), and *m/z* 635 ($n = 14$; Figure 3e), and the PEG/lamivudine complex ions at *m/z* 732 ($n = 11$; Figure 3f), and *m/z* 864 ($n = 14$; Figure 3g) show the mobility separations achieved. A general trend of decreasing mobility with increasing mass (corresponding to an increase in the ion collisional cross section) is observed. The resolution of IMS separations attained using our prototype IMS-Q-TOF spec-

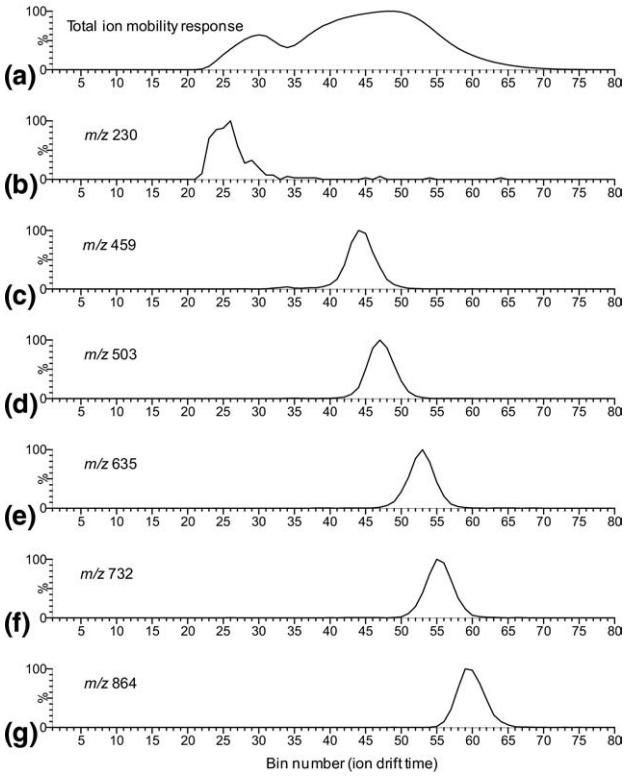

Figure 3. Ion mobility spectrum and selected ion mobility responses for a mixture containing lamivudine (10 μg mL^{-1}) and PEG 400 (1000 μg mL^{-1}); (**a**) total ion mobility spectrum, (**b**) protonated lamivudine (*m/z* 230), (**c**) co-drifting lamivudine dimer and a protonated PEG ion (*m/z* 459, $n = 10$), (**d**) protonated polyethylene glycol (*m/z* 503; $n = 11$), (**e**) protonated polyethylene glycol (*m/z* 635; $n = 14$), (**f**) lamivudine/PEG complex ion (*m/z* 732; $n = 11$), and (**g**) lamivudine/PEG complex ion (*m/z* 864; $n = 14$).

trometer compares well with commercial instruments (FWHH resolution ≈ 10–15), but it is unable to mobility resolve the protonated PEG oligomer (n = 10, m/z 459) and lamivudine dimer (m/z 459) responses, demonstrated by the single broad peak in the selected ion mobility spectrum of m/z 459 (Figure 3c). The broad ion mobility peak for m/z 230 suggests that the protonated lamivudine may be only partially separated from an excipient ion. The lamivudine/PEG complex ions have lower mobilities (longer drift times) than the protonated and cationized PEG oligomers because of their larger collision cross sections, and are fully resolved from the protonated lamivudine ion. The drift times for $[L + PEG + H]^+$ and $[PEG + H]^+$ converge as the PEG oligomer size (n) increases (Figure 2) and, as a consequence, the resulting mobility responses are less well resolved. For example, the separation of the $[L + PEG + H]^+$ and $[PEG + H]^+$ (m/z 732 and 503, respectively) for the n = 11 oligomer chain length is 0.52 ms (8 bins), whereas the separation of the $[L + PEG + H]^+$ and $[PEG + H]^+$ (m/z 864 and 635, respectively) for the n = 14 oligomer is 0.39 ms (6 bins). These data demonstrate that as the length of the PEG chain increases, the contribution to collisional cross section from the protonated lamivudine ion decreases. However, even the highest m/z observed for $[L + PEG + H]^+$ (m/z 1085, n = 19; t_d 4.36 ms) is still partially mobility-resolved from the corresponding $[PEG + H]^+$ (m/z 856, n = 19; t_d 3.97).

The selected ion mobility spectrum of the lamivudine/PEG complex ion at m/z 864 (n = 14; Figure 3g) shows a large shift in mass ($\Delta m/z$ = 634) and mobility (Δt_d = 2.3 ms) compared with the protonated lamivudine (m/z 230; Figure 3b). The response for lamivudine is therefore shifted through complexation with PEG oligomers, which is functioning as a "shift reagent", to a mass/mobility locus relatively free from strong interfering PEG responses [indicated by (filled star) in Figure 2]. Ions of the $[L + PEG + H]^+$ series were preselected by mass-to-charge using the quadrupole mass filter before entering the collision cell. Tandem mass spectrometry of the noncovalent complex at m/z 864 (n = 14; Figure 4) was performed using CID to recover the protonated lamivudine (m/z 230), as well as a lamivudine fragment (m/z 112), and the protonated PEG oligomer (n = 14; m/z 635). Ion mobility spectra were acquired whilst tandem mass spectrometry (CID) experiments were performed. The selected ion mobility response for the protonated lamivudine therefore has a drift time (t_d = 3.90 ms; bin number 60; Figure 4 insert) corresponding to the $[L + PEG + H]^+$ (m/z 864, n = 14) precursor complex ion, but with an m/z corresponding to $[L + H]^+$ (m/z 230). The position of the recovered m/z 230 product ion, $[L + H]^+$, is indicated by (filled circle) in Figure 2 and is located in a mobility and m/z area free from interfering PEG ions.

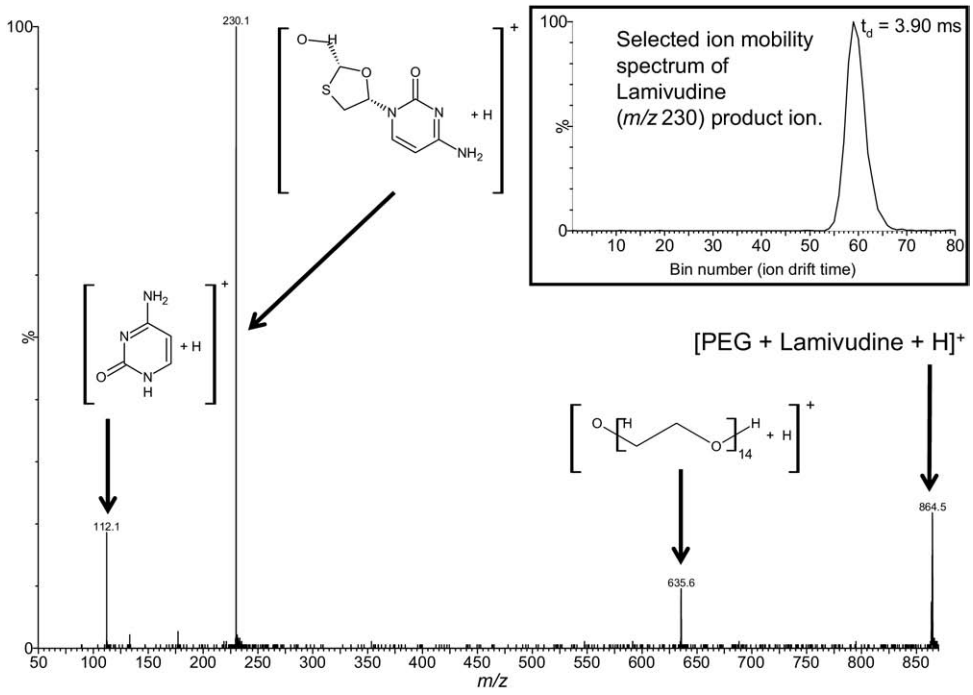

Figure 4. Tandem mass spectrum (CID) of lamivudine/PEG complex ion (m/z 864, n = 14) obtained from the ESI-IMS-Q-(CID)-TOF analysis of a lamivudine/PEG mixture with the quadrupole mass filter set pass only m/z 864 showing a lamivudine fragment (m/z 112), protonated lamivudine (m/z 230), protonated PEG (m/z 635) derived from the precursor lamivudine/PEG complex (m/z 864; n = 14), and (insert) the ion mobility spectrum of the recovered protonated lamivudine (m/z 230) product ion at the drift time corresponding to the lamivudine/PEG complex (m/z 864, n = 14; 3.90 ms).

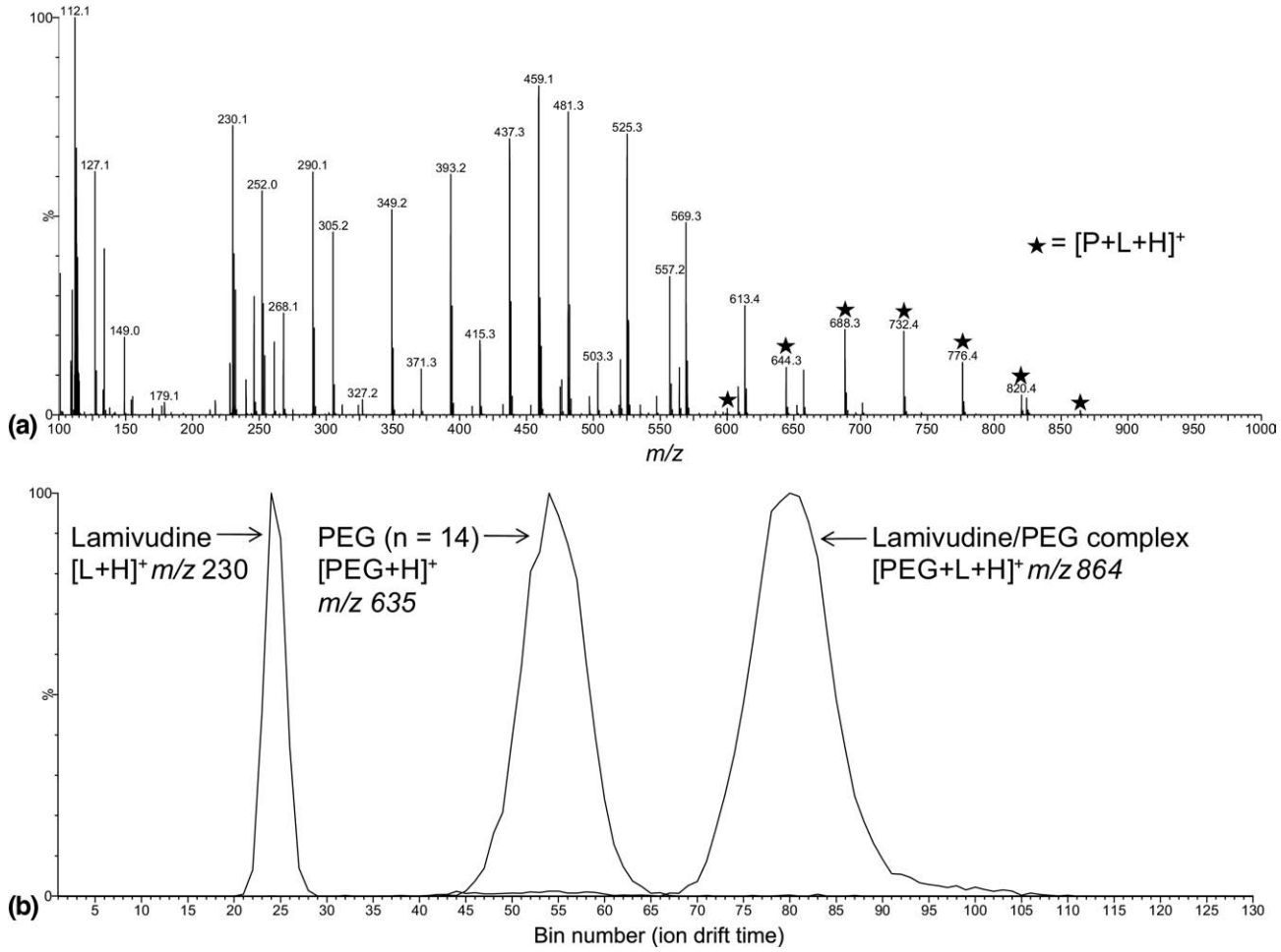

Figure 5. IM-MS data obtained from an electrosprayed infusion of a Combivir tablet, diluted 100-fold in 49.5/49.5/1 (vol/vol/vol) methanol/water/formic acid, acquired using a Waters Synapt spectrometer showing (**a**) the mass spectrum obtained by combining all 200 bins acquired during acquisitions of the ion mobility separation with $[L + PEG + H]^+$ complexes indicated by (filled star), (**b**) the selected ion mobility responses for lamivudine (*m/z* 230), PEG (*m/z* 635, $n = 14$), and a lamivudine/PEG complex (*m/z* 864, $n = 14$) overlaid with normalized intensities, obtained from a tandem MS experiment with the pusher pulse set to 45 μs and the quadrupole mass filter set to pass only *m/z* 864, followed by partial CID dissociation in the trap before mobility separation.

Analysis of a Pharmaceutical Formulation Containing lamivudine

The experiments on complexation of lamivudine with PEG were repeated for a Combivir tablet extract, which contained lamivudine in the presence of PEG excipients, using a Waters Synapt spectrometer. Figure 5a shows the mass spectrum, produced after combining all 200 mobility bins, showing lamivudine/PEG complex $[L + PEG + H]^+$ formation (indicated by a series of stars). Figure 5b shows the normalized selected mobility responses for lamivudine (*m/z* 230), PEG (*m/z* 635, $n = 14$), and the lamivudine/PEG complex (*m/z* 864, $n = 14$) acquired with the quadrupole filter positioned between the ESI source and the ion mobility drift cell set to pass only *m/z* 864. The lamivudine and PEG ions result from CID of the lamivudine/PEG complex before mobility separation. It is clear from Figure 5 that the protonated lamivudine, PEG, and lamivudine/PEG complex ions are fully mobility-resolved. For example, enhanced mobility separation is achieved through the shift in drift time for lamivudine to 3.80 ms (bin number 80) as a result of complexation with PEG ($n = 14$). Any of the PEG/lamivudine complexes may be chosen to recover the free protonated lamivudine and to maximize sensitivity the highest intensity complex should be isolated. However, the best selectivity may be achieved by selecting a higher mass complex such as the *m/z* 864 ($n = 14$). Figure 6 shows IM-MS data for the lamivudine/PEG complex (*m/z* 688, $n = 10$), in which the selected ions were subjected to partial fragmentation both before IM separation, in the trapping region before the drift cell, and in the transfer region after separation in the drift cell (i.e., ESI-MS-CID-IMS-CID-MS). Figure 6b shows the tandem mass spectrum produced when the lamivudine/PEG complex (*m/z* 688, $n = 10$) was fragmented in the transfer region after mobility separa-

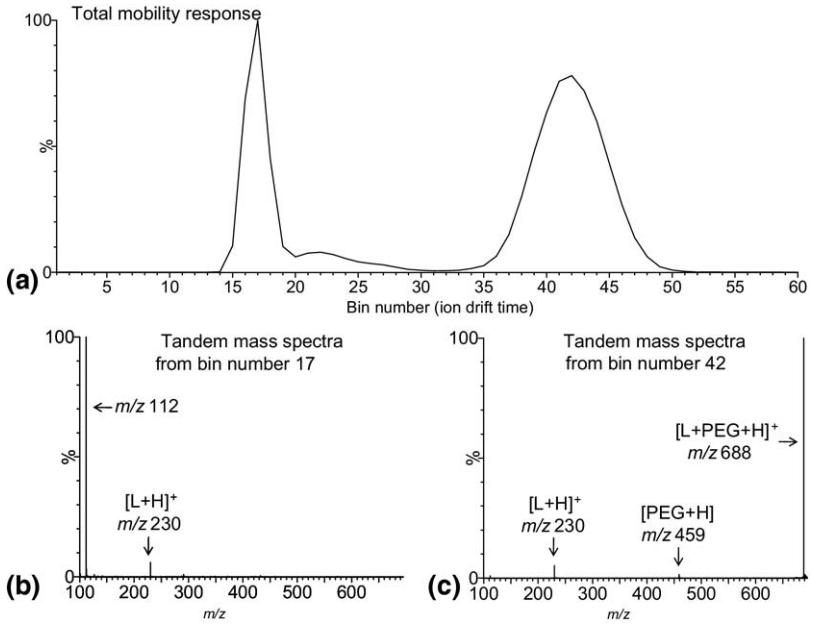

Figure 6. ESI-MS-CID-IMS-CID-MS spectrum of lamivudine/PEG complex ion (*m/z* 688, $n = 10$) obtained from an electrosprayed infusion of a Combivir tablet, diluted 100-fold in 49.5/49.5/1 (vol/vol/vol) methanol/water/formic acid, acquired using a Synapt mass spectrometer with the pusher pulse set to 65 μs showing (**a**) the total ion mobility response, (**b**) tandem MS spectrum obtained at t_d 2.78 ms (bin number 42) demonstrating post-drift cell (transfer region) fragmentation of lamivudine/PEG complex ions (*m/z* 688, $n = 10$) to yield protonated lamivudine (*m/z* 230) with a shifted drift time associated with the PEG/lamivudine complex, (**c**) MS/MS spectrum obtained at t_d 1.11 ms (bin number 17) demonstrating pre-drift cell (trap region) fragmentation of lamivudine/PEG complex (*m/z* 688) to yield protonated lamivudine (*m/z* 230) and a lamivudine fragment ion (*m/z* 112) with a drift time corresponding to the free lamivudine ion.

tion and, thus, the protonated lamivudine is associated with the longer drift time (1.89 ms; bin number 42) of the PEG/lamivudine complex. Figure 6c shows the tandem mass spectrum of the lamivudine/PEG complex (*m/z* 688, $n = 10$) fragmented before entering the drift cell, where protonated lamivudine released from the $[L + P + H]^+$ complex ion passed through the IMS cell and was fragmented in the transfer region to yield a characteristic fragment ion at *m/z* 112 with a drift time of 0.77 ms (bin number 17). Using the combination of in-trap CID, IMS drift cell separation, and CID in the transfer region of the Synapt spectrometer provides drift time measurements and tandem mass spectra for the fragments of the precursor and fragment ions. The data presented in Figure 6 therefore demonstrate the additional increase in selectivity for the IM-MS analyses using pre- and post-drift cell fragmentation.

Conclusions

A prototype IMS-Q-TOF spectrometer utilizing an ESI source has been used to study the gas-phase noncovalent complexes of an API, lamivudine, with PEG oligomers. A series of noncovalent complex ions containing the API and PEG were identified using tandem mass spectrometry. The protonated basic API has a decreased mobility when present as a noncovalent complex with a linear polyether. The potential of linear PEGs to act as internal shift reagents to enhance the separation and selectivity is demonstrated using a pharmaceutical formulation containing PEG with lamivudine. The free protonated lamivudine was recovered by tandem mass spectrometry with a drift time associated with the precursor complex. Recovery of the API from the API/shift reagent complex, by CID confirms the identity of the API and offers the potential for qualitative detection. Polyethers have the potential to be used as shift reagents when they are present in a formulation or could be added before the IM-MS analysis of an API, providing a method to enhance selectivity without modification of the ion mobility-mass spectrometer. Shift reagents may also be used for analyzing complex mixtures containing basic APIs, using stand alone IMS instrumentation. However, to avoid false positive results a lamivudine/PEG complex containing a single high molecular weight PEG oligomer or an additional shift reagent should be selected, to shift the mobility of the complex to an area free from interferences.

Acknowledgments

The authors acknowledge GlaxoSmithKline and the Engineering and Physical Sciences Research Council for financial support.

References

1. Eiceman, G. A.; Karpas, Z. *Ion Mobility Spectrometry*, 2nd ed.; CRC Press: Boca Raton (FL) 2005.
2. Borsdorf, H.; Eiceman, G. A. Ion Mobility Spectrometry: Principles and Applications. *Appl. Spectrosc. Rev.* **2006,** *41,* 323–375.
3. Karpas, Z.; Eiceman, G. A.; Krylov, E. V.; Krylova, N. Models on Ion Heating and Mobility in Linear Field Drift Tubes and in Differential Mobility Spectrometers. *Int. J. Ion Mobility Spectrom.* **2004,** *7,* 8–18.
4. Eiceman, G. A. Ion-Mobility Spectrometry as a Fast Monitor of Chemical Composition. *TrAC Trends Anal. Chem.* **2002,** *21,* 259–275.
5. Creaser, C. S.; Griffiths, J. R.; Bramwell, C. J.; Noreen, S.; Hill, C. A.; Thomas, C. L. P. Ion Mobility Spectrometry: A Review. Part 1. Structural Analysis by Mobility Measurement. *Analyst* **2004,** *129,* 984–994.
6. Revercomb, H. E.; Mason, E. A. Theory of Plasma Chromatography/Gaseous Electrophoresis—A Review. *Anal. Chem.* **1975,** *47,* 970–983.
7. O'Donnell, R. M.; Sun, X.; Harrington, P. D. B. Pharmaceutical Applications of Ion Mobility Spectrometry. *TrAC Trends Anal. Chem.* **2008,** *27,* 44–53.
8. Budimir, N.; Weston, D. J.; Creaser, C. S. Analysis of Pharmaceutical Formulations Using Atmospheric Pressure Ion Mobility Spectrometry Combined with Liquid Chromatography and Nano-Electrospray Ionization. *Analyst* **2007,** *132,* 34–40.
9. Wang, Y.; Nacson, S.; Pawliszyn, J. The Coupling of Solid-Phase Microextraction/Surface Enhanced Laser Desorption/Ionization to Ion Mobility Spectrometry for Drug Analysis. *Anal. Chim. Acta* **2007,** *582,* 50–54.
10. Lokhnauth, J. K.; Snow, N. H. Determination of Parabens in Pharmaceutical Formulations by Solid-Phase Microextraction-Ion Mobility Spectrometry. *Anal. Chem.* **2005,** *77,* 5938–5946.
11. Jafari, M. T.; Khayamian, T.; Shaer, V.; Zarei, N. Determination of Veterinary Drug Residues in Chicken Meat Using Corona Discharge Ion Mobility Spectrometry. *Anal. Chim. Acta* **2007,** *581,* 147–153.
12. Jafari, M. T. Determination and Identification of Malathion, Ethion, and Dichlorvos Using Ion Mobility Spectrometry. *Talanta* **2006,** *69,* 1054–1058.
13. Ochoa, M. L.; Harrington, P. B. Detection of Methamphetamine in the Presence of Nicotine Using *in situ* Chemical Derivatization and Ion Mobility Spectrometry. *Anal. Chem.* **2004,** *76,* 985–991.
14. Kudriavtseva, S.; Carey, C.; Ribeiro, K.; Wu, C. Detection of Drugs of Abuse in Sweat Using Ion Trap Mobility Spectrometry. *Int. J. Ion Mobility Spectrom.* **2004,** *7,* 44–51.
15. Kanu, A. B.; Hill, H. H. Identity Confirmation of Drugs and Explosives in Ion Mobility Spectrometry Using a Secondary Drift Gas. *Talanta* **2007,** *73,* 692–699.
16. Khayamian, T.; Tabrizchi, M.; Jafari, M. T. Quantitative Analysis of Morphine and Noscapine Using Corona Discharge Ion Mobility Spectrometry with Ammonia Reagent gas. *Talanta* **2006,** *69,* 795–799.
17. Kanu, A. B.; Dwivedi, P.; Tam, M.; Matz, L.; Herbert H. Hill, J. Ion Mobility-Mass Spectrometry, *J. Mass Spectrom.* **2008,** *43,* 1–22.
18. Weston, D. J.; Bateman, R.; Wilson, I. D.; Wood, T. R.; Creaser, C. S. Direct Analysis of Pharmaceutical Drug Formulations Using Ion Mobility Spectrometry/Quadrupole-Time-of-Flight Mass Spectrometry Combined with Desorption Electrospray Ionization. *Anal. Chem.* **2005,** *77,* 7572–7580.
19. Eckers, C.; Laures, A.M.-F.; Giles, K.; Major, H.; Pringle, S. Evaluating the Utility of Ion Mobility Separation in Combination with High-Pressure Liquid Chromatography/Mass Spectrometry to Facilitate Detection of Trace Impurities in Formulated Drug Products. *Rapid Commun. Mass Spectrom.* **2007,** *21,* 1255–1263.
20. Matz, L. M.; Hill, H. H. Separation of Benzodiazepines by Electrospray Ionization Ion Mobility Spectrometry-Mass Spectrometry. *Anal. Chim. Acta*, **2002,** *457,* 235–245.
21. Griffin, L. B. S. Trace Level Confirmation of Controlled Substances Found by Ion Mobility Spectrometry, with Quadrupole Ion-Trap Mass Spectrometry. *Int. J. Ion Mobility Spectrom.* **2002,** *5,* 31–40.
22. Wu, C.; Siems, W. F.; Hill, H. H. Secondary Electrospray Ionization Ion Mobility Spectrometry/Mass Spectrometry of Illicit Drugs. *Anal. Chem.* **2000,** *72,* 396–403.
23. Matz, L. M.; Hill, H. H. Evaluation of Opiate Separation by High-Resolution Electrospray Ionization-Ion Mobility Spectrometry/Mass Spectrometry. *Anal. Chem.* **2001,** *73,* 1664–1669.
24. Dugourd, P.; Hudgins, R. R.; Clemmer, D. E.; Jarrold, M. F. High-Resolution Ion Mobility Measurements. *Rev. Sci. Instrum.* **1997,** *68,* 1122–1129.
25. Tabrizchi, M. Temperature Effects on Resolution in Ion Mobility Spectrometry. *Talanta* **2004,** *62,* 65–70.
26. Thackston, M. G.; Ellis, H. W.; Pai, R. Y.; McDaniel, E. W. Mobilities of Rubidium(+) Ions in Helium, Neon, Argon, Hydrogen, Nitrogen, Oxygen, and Carbon Dioxide. *J. Chem. Phys.* **1976,** *65,* 2037–2038.
27. Ruotolo, B. T.; McLean, J. A.; Gillig, K. J.; Russell, D. H. Peak Capacity of Ion Mobility Mass Spectrometry: The Utility of Varying Drift Gas Polarizability for the Separation of Tryptic Peptides. *J. Mass Spectrom.* **2004,** *39,* 361–367.
28. Asbury, G. R.; Herbert H. Hill, J. Using Different Drift Gases to Change Separation Factors (α) in Ion Mobility Spectrometry. *Anal. Chem.* **2000,** *72,* 580–584.
29. Creaser, C. S.; Griffiths, J. R.; Stockton, B. M. Gas-Phase Ion Mobility Studies of Amines and Polyether/Amine Complexes Using Tandem Quadrupole Ion Trap/Ion Mobility Spectrometry. *Eur. J. Mass Spectrom.* **2000,** *6,* 213–218.
30. Creaser, C. S.; Benyezzar, M.; Griffiths, J. R.; Stygall, J. W. A Tandem Ion Trap/Ion Mobility Spectrometer. *Anal. Chem.* **2000,** *72,* 2724–2729.
31. Creaser, C. S.; Griffiths, J. R.; Stockton, B. M. In *Advances in Mass Spectrometry 2001* Wiley: Chichester, Vol. XV; p. 407–408.
32. Creaser, C. S.; Griffiths, J. R. Atmospheric Pressure Ion Mobility Spectrometry Studies of Cyclic and Acyclic Polyethers. *Anal. Chim. Acta* **2001,** *436,* 273–279.
33. Colgrave, M. L.; Bramwell, C. J.; Creaser, C. S. Nanoelectrospray Ion Mobility Spectrometry and Ion Trap Mass Spectrometry Studies of the Noncovalent Complexes of Amino Acids and Peptides with Polyethers. *Int. J. Mass Spectrom.* **2003,** *229,* 209–216.
34. Hilderbrand, A. E.; Myung, S.; Clemmer, D. E. Exploring Crown Ethers as Shift Reagents for Ion Mobility Spectrometry. *Anal. Chem.* **2006,** *78,* 6792–6800.
35. Clowers, B. H.; Herbert, H.; Hill, J. Influence of Cation Adduction on the Separation Characteristics of Flavonoid Diglycoside Isomers Using Dual Gate-Ion Mobility-Quadrupole Ion Trap Mass Spectrometry. *J. Mass Spectrom.* **2006,** *41,* 339–351.
36. Dwivedi, P.; Bendiak, B.; Clowers, B. H.; Hill, H. H. Jr. Rapid Resolution of Carbohydrate Isomers by Electrospray Ionization Ambient Pressure Ion Mobility Spectrometry-Time-of-Flight Mass Spectrometry (ESI-APIMS-TOFMS). *J. Am. Soc. Mass Spectrom.* **2007,** *18,* 1163–1175.
37. Kerr, T. J.; McLean, J. A. Functionally Selective Ion Mobility Shift Reagents for Proteomic Applications. *Proceedings of the 55th ASMS Conference on Mass Spectrometry and Allied Topics*; Indianapolis, IN, June 2007.
38. Vonhelden, G.; Wyttenbach, T.; Bowers, M. T. Conformation of Macromolecules in the Gas-Phase—Use of Matrix-Assisted Laser-Desorption Methods in Ion Chromatography. *Science* **1995,** *267,* 1483–1485.
39. Vonhelden, G. V.; Wyttenbach, T.; Bowers, M. T. Inclusion of a MALDI Ion-Source in the Ion Chromatography Technique—Conformational Information on Polymer and Biomolecular Ions. *Int. J. Mass Spectrom. Ion Processes* **1995,** *146,* 349–364.
40. Wyttenbach, T.; von Helden, G.; Batka, J. J. Jr.; Carlat, D.; Bowers, M. T. Effect of the Long-Range Potential on Ion Mobility Measurements. *J. Am. Soc. Mass Spectrom.* **1997,** *8,* 275–282.
41. Gidden, J.; Wyttenbach, T.; Jackson, A. T.; Scrivens, J. H.; Bowers, M. T. Gas-Phase Conformations of Synthetic Polymers: Poly(Ethylene Glycol), Poly(Propylene Glycol), and Poly(Tetramethylene Glycol). *J. Am. Chem. Soc.* **2000,** *122,* 4692–4699.
42. Wyttenbach, T.; von Helden, G.; Bowers, M. T. Conformations of Alkali Ion Cationized Polyethers in the Gas Phase: Polyethylene Glycol and Bis[(Benzo-15-Crown-5)-15-Ylmethyl] Pimelate. *Int. J. Mass Spectrom. Ion Processes* **1997,** *165/166,* 377–390.
43. Krylov, E. V.; Nazarov, E. G.; Miller, R. A. Differential Mobility Spectrometer: Model of Operation. *Int. J. Mass Spectrom.* **2007,** *266,* 76–85.
44. Kolakowski, B. M.; Mester, Z. Review of Applications of High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) and Differential Mobility Spectrometry (DMS). *Analyst* **2007,** *132,* 842–864.
45. Ude, S.; Mora, de la Mora, J. F.; Thomson, B. A. Charge-Induced Unfolding of Multiply Charged Polyethylene Glycol ions. *J. Am. Chem. Soc.* **2004,** *126,* 12184–12190.
46. Pringle, S. D.; Giles, K.; Wildgoose, J. L.; Williams, J. P.; Slade, S. E.; Thalassinos, K.; Bateman, R. H.; Bowers, M. T.; Scrivens, J. H. An Investigation of the Mobility Separation of Some Peptide and Protein Ions Using a New Hybrid Quadrupole/Travelling Wave IMS/oa-TOF Instrument. *Int. J. Mass Spectrom.* **2007,** *261,* 1–12.
47. Lee, S.; Wyttenbach, T.; Vonhelden, G.; Bowers, M. T. Gas-Phase Conformations of Li^+, Na^+, K^+, and Cs^+ complexed with 18-Crown-6. *J. Am. Chem. Soc.* **1995,** *117,* 10159–10160.
48. Kaur-Atwal, G.; Weston, D. J.; Green, P. S.; Crosland, S.; Bonner, P. L. R.; Creaser, C. S. Analysis of Tryptic Peptides Using Desorption Electrospray Ionization Combined with Ion Mobility Spectrometry/Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2007,** *21,* 1131–1138.
49. Vakhrushev, S. Y.; Langridge, J.; Campuzano, I.; Hughes, C.; Peter-Katlinic, J. Ion Mobility Mass Spectrometry Analysis of Human Glycourinome. *Anal. Chem.* **2008,** *80,* 2506–2513.