RESEARCH ARTICLE

Predicting Compensation Voltage for Singly-charged Ions in High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS)

Alexander A. Aksenov,[1,3] James Kapron,[2] Cristina E. Davis[3]

[1]LGC Ltd, Teddington, Middlesex, TW11 0LY, UK
[2]SciIns Marketing, Ottawa, Canada
[3]Department of Mechanical and Aerospace Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

Abstract

Correlation between compensation voltage (CV) and the *m/z* ratio of singly-charged ions was elucidated. The experimental data for various alkylammonium homologues and various pharmaceutical compounds were used to construct empirical calibration curves that were fit using commercial regression analysis software packages. The best fit equations were applied to calculate the CV differences (ΔCV) in pure N_2 and N_2/He 50/50 carrier gasses and CV values for a variety of compounds using only *m/z* values. The calculated values were in good agreement with experimental data and ΔCV values exhibited a very strong correlation with *m/z*. Application of these empirical calculations may provide a powerful CV prediction tool for researchers using high-field asymmetric waveform ion mobility spectrometry (FAIMS) and increase the value of FAIMS as an analytical method.

Key words: High field asymmetric waveform ion mobility spectrometry (FAIMS), Differential mobility spectrometry (DMS), Compensation voltage (CV), Mass to charge ratio (*m/z*), Mathematical prediction models

Introduction

The technology of high-field asymmetric waveform ion mobility spectrometry (FAIMS), often called Differential Mobility Spectrometry (DMS) [1] originated in the USSR during the early 1980s [2]. FAIMS/MS is able to resolve small-molecule signals from nearly-isobaric interferences, and it reduces the chemical noise generated in the ion source of a mass spectrometer, improving selectivity and quantitative accuracy [3]. The technology exploits differences in ion mobility at high and low electric fields [4]. A DC potential called a compensation voltage (CV) is applied to a FAIMS electrode to compensate for ion drift under varying field conditions and allow a subset of ions to pass through the device. The CV value reflects an ion's properties as it relates to mobility under differential field conditions and therefore is unique to each ion. The CV values can be used to identify certain ions or to allow ion filtering in a FAIMS device prior to introduction into the MS [5]. However, challenges arise in linking CV values to ion identities. In particular, it is not always possible to predict an ion's CV. The difficulty is mainly due to non-linear dependence of the ion's mobility with the field strength, which is not always known for an ion/gas combination.

Previously, successful attempts have been made to design an appropriate waveform and predict the CV for simple ions [6, 7]. For the majority of ions in a single buffer gas or in gas mixtures, such predictions are not possible because the

dependence of mobility on the electric field strength is not known. For practical applications, it is customary to infuse a pure standard and ramp the CV to experimentally determine the compound's corresponding CV. For subsequent FAIMS experiments (most often as FAIMS/MS or LC/FAIMS/MS), the CV is then set for the previously determined conditions. For example, in drug metabolism studies, researchers would like to exclude chemical noise, but be assured that all metabolites will be included. If the CV values of metabolites are not known, such an experiment will require scanning of the entire CV space, prolonging the FAIMS scan and affecting total duty cycle.

Certain system designs allow for short ion residence times within the FAIMS device, which enables faster waveform scans [8]. For designs of FAIMS devices where fast scan is not possible, the best way to increase throughput is to estimate the CV values prior to the experiment and then scan only the informative part of the CV space. The prediction of CV is the major intellectual challenge to the successful application of this technology in the modern laboratory.

It is known that ion mobility correlates with ion size; however, the correlation to the ion mass or m/z is generally weaker for differential mobility (ΔK) than for K (i.e., FAIMS is more orthogonal to MS than conventional IMS [9, 10]). It was also shown with the use of buffer gas modifiers [11] that complexation plays a certain role in the order of emergence and resolution of ions within FAIMS systems. In general, reports of correlation of CV and m/z are scarce and sometimes conflicting. For example, for CV–m/z correlations for triazine pollutant compounds [12], and in homologues series of lipids [13] have opposing trends reported.

In the present work, we take the approach that upon elimination of complicating factors such as solvation, CV values for singly-charged "reference" ions can be used to create a plot of CV and ΔCV versus m/z. Unknown ΔCV and CV values for other compounds can then be determined based solely upon m/z of ions.

Experimental

Chemicals and Reagents

All chemicals were purchased from Sigma Aldrich (Gillingham, UK). The pharmaceuticals were: codeine, terfenadine, reserpine, and erythromycin ethyl succinate. The amine compounds were: triethylamine, N,N-dimethylbutylamine, N,N-dimethylhexylamine, tetramethyl ammonium bromide hexadecyltrimethylammonium bromide, and ethanolamine. The tetraalkyl ammonium halides (TAA) compounds were NR4X (where R=methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, and n-decyl, and X=Br, I). The drift time ion mobility spectrometry (DT IMS) calibrants for positive ion were dimethyl methylphosphonate (DMMP) and lutidine. DT IMS calibrants for negative ions were taur-

ocholic acid and dipropylene glycol (DPG). In addition, the FAIMS system performance check compounds (polytyrosine, 1-, 3-, and 6-isomers) supplied along with the FAIMS system by Thermo Fisher Scientific (San Jose, CA, USA) were used. HPLC-grade acetonitrile (ACN), methanol (MeOH) and formic acid were obtained from commercial sources and used without further purification. Industrial grade gases and deionized water were used throughout.

Sample Preparation and FAIMS/MS Analysis

Stock solutions of each compound were prepared by weighing the material and dissolving in ACN to 1 mg/mL. Combined solutions of the TAAs were prepared by mixing individual TAA solutions. The FAIMS/MS analysis is described in detail elsewhere [14]. All the experiments were conducted using direct ESI infusion at 5 µL/min flow rate. The FAIMS/MS system TSQ Quantum Ultra (Thermo Fisher Scientific, San Jose, CA, USA) recommended standard conditions were used throughout (positive ions): dispersion voltage (DV) –5 kV, 3.5 L/min equimolar nitrogen and helium carrier gas, inner electrode temperature 70 °C, and outer electrode temperature 90 °C. Heated electrospray ionization (H-ESI) parameters were set as follows: ion spray voltage 4000 V, vaporizer temperature 400 °C, tube lens offset 100 V.

Curve Fitting

Microsoft Excel (Redmond, WA, USA) software was used for fitting of ΔCV plots. Systat SigmaPlot™ (Chicago, IL, USA) software was used in all other cases. Multiple fitting functions were tested. The best fitting function which produced the highest coefficient of determination (R^2) value was then evaluated with respect to its performance on experimental data.

Results and Discussion

Care was taken to ensure that the FAIMS instrument was operated under conditions that produce desolvated ions in order to eliminate irreproducibility issues associated with the effects caused by ion solvation/clustering. Reproducibility

Table 1. CV Values for Several Pharmaceutical and Amine/Ammonium Compounds

Compound	Q1 (m/z)	CV(50), V	CV(0), V
Codeine	300	–14.8	–5.7
Terfenadine	472	–15.9	–9.1
Reserpine	609	–12.1	–6.3
Erthyromycin ethyl succinate – water	844	–11.9	–6.9
Triethylamine	102	–15.8	7.6
N,N-dimethylbutylamine	102	–15.8	7.6
N,N-dimethylhexylamine	130	–14.3	1.6
Hexadecyltrimethylammonium	284	–21.5	–11.9

Table 2. CV Values for TAA Homologues

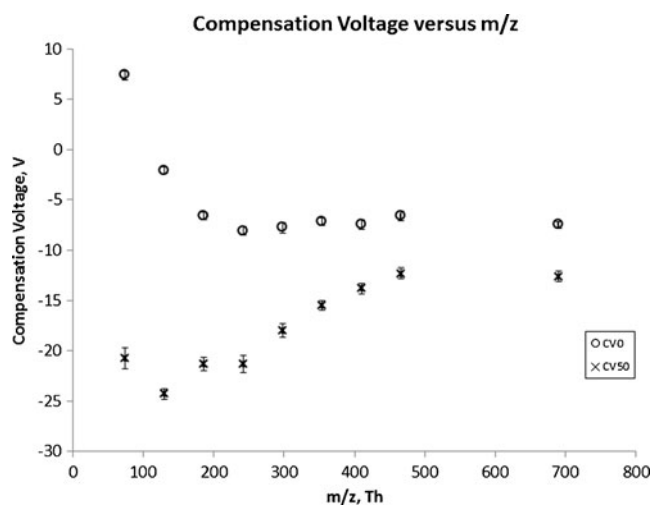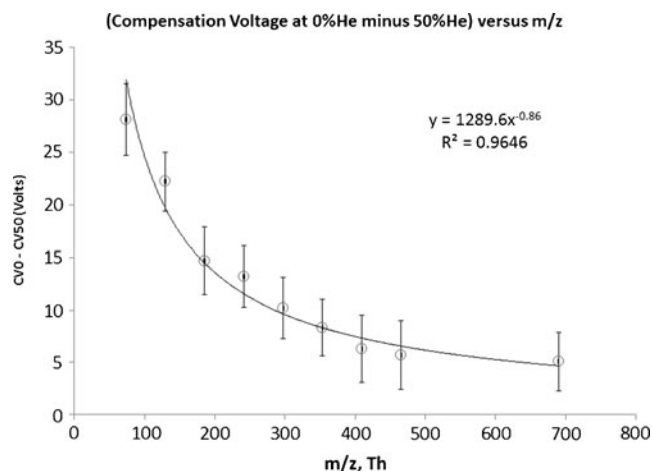| Cation	Q1 (m/z)	CV(50), V	CV(0), V
Tetramethylammonium	74	−20.1	7.4
Tetraethylammonium	130	−24.3	−2.4
Tetrapropylammonium	186	−21.3	−6.6
Tetrabutylammonium	242	−21.3	−8.2
Tetrapentylammonium	298	−18.3	−7.8
Tetrahexylammonium	354	−15.3	−7.2
Tetraheptylammonium	410	−14.1	−7
Tetraoctylammonium	466	−12.6	−6.2
Tetradodecylammonium	690	−12.6	−7

within a few tenths of a volt was routinely achieved for desolvated ions.

The CV values of several tested pharmaceuticals and trialkyl amine compounds are listed in Table 1; the values for tetraalkylammonium cations are listed in Table 2. The notation CV(0) represents the compensation voltages in pure nitrogen, and CV(50) represents the compensation voltages in the N2/He 50:50 gas mix. The relative transmission of CV peaks for the pharmaceutical compounds remained unaltered when the percentage of He is increased. In contrast, great differences in FAIMS behavior were observed for small and large TAA homologues. The behavior of TAA ions has been studied previously in some detail [14].

The CV(0) and CV(50) values plotted versus m/z for compounds listed in Tables 1 and 2 are shown in Figure 1a. It can be seen that the CV values exhibit trends with m/z for both the homologous series and the other ions. Trends appear more pronounced for CV(0) values.

The differences in "geometry factor" stemming from differences in ion size, charge distribution variations, and other factor will lead to differences in the ion–neutral interactions as evidenced by point scatter in the CV-m/z plots seen in Figure 1.

For the gasses that do not cluster with gas-phase ions, such as the ones used in this study, variations of the buffer gas composition are not expected to affect the gas-phase structure

Figure 2. ΔCV fit generated for compounds in Tables 1 and 2; the error bars represent FWHM of the peak

of the ions. Therefore, ΔCV values obtained by subtracting CV values in two different gases (ΔCV=CVGAS1 − CVGAS2), could be expected to be less influenced by the "geometry factor" of an ion, which will be mostly cancelled out by such subtraction. An example plot of ΔCV at 0 % and 50 % He in nitrogen [CV(0) – CV(50)] for all ions in Tables 1 and 2 is shown in Figure 2. Indeed, the point scatter is greatly reduced compared to the CV – m/z plots shown in Figure 1. The ΔCV value can be called a "CV variance capacity" for carrier gas modification: it is the magnitude of CV shift which an ion undergoes due to change of gas mixture composition. The slope of the ΔCV versus m/z curve is greatest at low m/z values, which indicates that the smaller ions will exhibit greater response to the gas composition change than larger ones. This behavior was described for homologous series of TAA ions [14].

The power fit equation $y = ax^b$ provides the best fit for the curve shown in Figure 2:

$$\Delta CV = 757.67 \cdot (m/z)^{0.7605}$$
$$R^2 = 0.9646 \tag{1}$$

Figure 1. CV values of compounds in Tables 1 and 2 in nitrogen (CV0) and 1:1 N2/He (CV50)
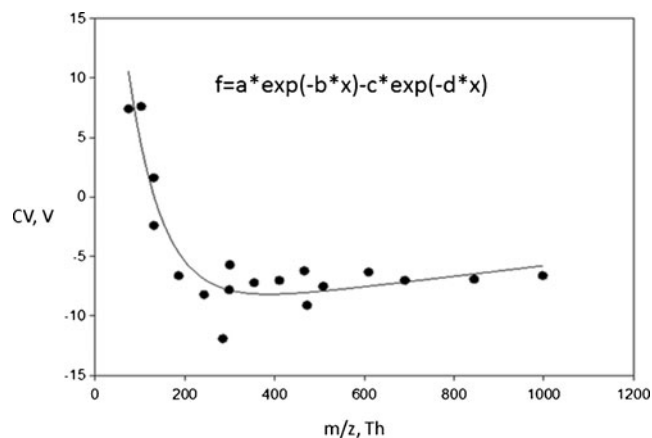

Figure 3. CV(0) versus m/z plot generated for compounds from Tables 1 and 2 with a fit curve (Equation 2)

Table 3. Comparison of Calculated and Experimental Values for ΔCV, CV(0) and CV(50)

Standards	Q1 (*m/z*)	ΔCV$_{exp}$, V	ΔCV$_{calc}$, V	ΔCV error	CV(50) Using CV(0)$_{exp}$	CV(50) Using CV(0)$_{exp}$ error	CV(0)$_{calc}$, V	CV(50) Using CV(0)$_{calc}$	CV(50) Using CV(0)$_{calc}$ error
Tetramethylammonium	74	27.5	28.7	4.5 %	–21.3	6.2 %	10.44	–18.3	–9.0 %
Tetraethylammonium	130	20.1	18.7	–6.9 %	–21.1	–6.1 %	0.1	–18.5	–18.0 %
Tetrapropylammonium	186	14.7	14.3	–3.0 %	–20.9	–2.1 %	–4.70	–18.9	–11.0 %
Tetrabutylammonium	242	13.1	11.7	– 10.9 %	–19.9	–6.7 %	–7.01	–18.7	–12.3 %
Tetrapentylammonium	298	10.5	10.0	–5.1 %	–17.8	–2.9 %	–7.95	–17.9	–2.1 %
Tetrahexylammonium	354	8.1	8.7	7.9 %	–15.9	4.2 %	–8.20	–16.9	10.7 %
Tetraheptylammonium	410	7.1	7.8	10.1 %	–14.8	5.1 %	–8.11	–15.9	12.9 %
Tetraoctylammonium	466	6.4	7.1	10.8 %	–13.3	5.5 %	–7.85	–14.9	18.6 %
Tetradodecylammonium	690	5.6	5.3	–6.1 %	–12.3	–2.7 %	–6.43	–11.7	–7.2 %
Triethylammonium + H	102	23.5	22.3	–4.2 %	–14.9	–6.2 %	4.48	–18.0	13.5 %
N,N-dimethylbutylammonium + H	102	23.5	22.3	–4.2 %	–14.9	–6.2 %	4.48	–18.0	13.5 %
N,N-dimethylhexylammonium + H	130	17.2	18.6	8.8 %	–17.1	9.8 %	0.27	–18.5	18.3 %
Hexadecyltrimethylammonium	284	9.6	10.3	7.6 %	–22.2	3.4 %	–7.80	–18.1	–15.7 %
Codeine + H	300	9.6	9.9	3.2 %	–15.6	2.0 %	–7.97	–17.9	16.9 %
Terfenadine + H	472	6.8	7.1	3.3 %	–16.1	1.4 %	–7.81	–14.9	–6.7 %
Reserpine + H	609	5.8	5.8	–0.3 %	–12.1	–0.1 %	–6.95	–12.7	5.3 %
Erythromycin ethyl succinyl + H	844	5	4.6	–9.7 %	–11.4	–4.1 %	–5.52	–10.0	–15.7 %

Calculations of CV variance capacity (ΔCV) may be beneficial for estimating the extent of CV shift resulting from gas composition changes. For a routine FAIMS analysis, the standard conditions are predominantly used and the CV(0) and CV(50) values are most commonly reported. Calculations of ΔCV values using the empirical Equation (1) will allow estimating the CV(50) value when the value of CV(0) is available (or vice versa).

To account for the "geometry factor," a variety of structurally unrelated compounds as well as homologues from Tables 1 and 2 were employed for building the calibration curve. The plotted CV(0) versus *m/z* values and a fitting curve are shown in Figure 3. The function that was found to produce the best fit to the data (i.e., providing the greatest R2 value) is:

$$CV = 53.5940e^{-0.0118m/z} - 12.8544e^{-0.0010m/z} \quad (2)$$

which resulted in an $R^2 = 0.8792$. For very large ions, the constraint $\lim_{m/z \to \infty} CV = 0$ was imposed for all tested functions.

The calculated and experimental CV values are listed in Table 3. The experimental CV(0) and CV(50) values listed in Tables 1 and 2 are labeled CV(0)exp and CV(50)exp, respectively, in Table 3.

In addition to "calibration" compounds, we also employed a set of independent test compounds not used in the prior equation fitting. Several compounds commonly used in ion mobility experiments such as DT IMS calibrants (lutidine, DMMP), the FAIMS system performance check compound (polytyrosine, 1-, 3-, and 6-isomers), as well as negatively charged ions (taurocholic acid, DPG) were employed as independent test compounds. The calculated and experimental CV values for the test compounds are presented in Table 4. As can be seen, the results are in line with those obtained for the "calibration" compounds. For the majority of both reference and test compounds, the calcu-

lated CV(50) values fall within 15 % of expected (experimental) values, and fall within less than 20 % error for all of the tested compounds. The error values are similar for both reference and test compounds with the error sign changing randomly, which indicates that no systemic bias of CV values occurs. The 15 % error means that for a compound at CV of – 10 V, a calculated value will fall in the range of –11.5 V to – 8.5 V. Scanning a window of only a few volts around a precalculated value can greatly reduce FAIMS scan times. This is significant for increased throughput, as many commercially available FAIMS instruments, such as the one used in the present work, performs a scan of 40 V over approximately 90 s.

It should be emphasized that the above considerations only apply when ions are properly desolvated [11]. As a troubleshooting aid, a discrepancy between predicted and experimental CV values may help to trace potential problems in experimental operation [4]. For example, a lack of desolvation or presence of chemical transformations may be indicated if the experimental value does not agree with the predicted value.

In this study, only singly-charged ions are considered. It is known that doubly and triply charged peptides emerge at CV values more negative than –20 V (at standard conditions) [9, 15]. Thus, Equations 1 and 2 cannot be applied

Table 4. Calculated and Experimental Values for CV(50) Values for Test Compounds not Used in Calibration Curves Generation

Testers	Q1 (*m/z*)	CV(50)$_{exp}$, V	CV(50) using CV(0)$_{calc}$, V	Error, %
Lutidine	107	–16.2	–18.5	14.1
DMMP	124	–20.1	–18.9	–6.2
DPG*	176	17.5	19.3*	10.4
Testosterone glucoronide	465	–15.6	–15.2	–2.6
Polytyrosine 3-mer	508	–15.3	–14.6	–4.8
Polytyrosine 6-mer	997	–11.1	–9.8	–11.8
Taurocholic acid*	514	16.6	14.5*	–12.7

*The CV values for negative ions were calculated by changing sign of CV (50) values calculated from the fitting equations

to multiply charged ions. In general, large proteins emerge at more negative CV values than those predicted by Equations 1 and 2.

The trends reported in this study are not absolute and the CV values will invariably differ for different ions with the same m/z due to unique ion structure, which leads to differences in gas-phase interactions and a corresponding unique dependence on K0(E/N). In fact, deviations of CV values from a single trend make FAIMS more valuable as an analytical method. For example, separation of leucine and isoleucine is possible with FAIMS [16]. The results of the present work, however, point out that these deviations of CV values occur on top of a general underlying trend that may be often obscured.

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