Development of atmospheric pressure ionization ion mobility spectrometry and ion mobility spectrometry – mass spectrometry

Alexey Adamov

Academic Dissertation

To be presented, with the permission of the Faculty of Science of the University of Helsinki, for public criticism in Auditorium A129 of the Department of Chemistry (A.I. Virtasen aukio 1, Helsinki) on January 13, 2012, at 12 noon.

Helsinki 2011
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ISBN 978-952-10-7563-6 (PDF)

http://ethesis.helsinki.fi

Unigrafia Oy
Helsinki 2011
Preface

This thesis is based on research carried out in the Laboratory of Analytical Chemistry of the Department of Chemistry and Division of Pharmaceutical Chemistry of the Faculty of Pharmacy, University of Helsinki, during the years 2003-2011. This research was financially supported by the Academy of Finland, the Finnish Funding Agency for Technology and Innovation (Tekes), and the University of Helsinki.

I am most grateful to my supervisors Professor Tapio Kotiaho and Associate Professor Alexey Sysoev for opportunity to carry out this work, encouragement, fresh ideas, positive attitude, and patience. I would like also to thank Professor Marja-Liisa Riekkola for helpful advises and help in my studies, and Professors Ari Ivaska and Ari Tolonen for carrying out a careful review of this manuscript.

I would like to thank former and present personal of the Laboratory of Analytical Chemistry, Department of Chemistry and Division of Pharmaceutical Chemistry, Faculty of Pharmacy for assistance, pleasant working atmosphere and leisure-time activities.

Above all, loving thanks to my parents and to all my friends for their support.

Helsinki, December 2011

Alexey Adamov
Abstract

This study is focused on the development and evaluation of ion mobility instrumentation with various atmospheric pressure ionization techniques and includes the following work.

First, a high-resolution drift tube ion mobility spectrometer (IMS), coupled with a commercial triple quadrupole mass spectrometer (MS), was developed. This drift tube IMS is compatible with the front-end of commercial Sciex mass spectrometers (e.g., Sciex API-300, 365, and 3000) and also allows easy (only minor modifications are needed) installation between the original atmospheric pressure ion source and the triple quadrupole mass spectrometer. Performance characteristics (e.g., resolving power, detection limit, transmission efficiency of ions) of this IMS-MS instrument were evaluated. Development of the IMS-MS instrument also led to a study where a proposal was made that tetraalkylammonium ions can be used as chemical standards for ESI-IMS.

Second, the same drift tube design was also used to build a standalone ion mobility spectrometer equipped with a Faraday plate detector. For this high-resolution (resolving power about 100 shown) IMS device, a multi-ion source platform was built, which allows the use of a range of atmospheric pressure ionization methods, such as: corona discharge chemical ionization (CD-APCI), atmospheric pressure photoionization (APPI), and radioactive atmospheric pressure chemical ionization (R-APCI). The multi-ion source platform provides easy switching between ionization methods and both positive and negative ionization modes can be used.

Third, a simple desorption/ionization on silicon (DIOS) ion source set-up for use with the developed IMS and IMS-MS instruments was built and its operation demonstrated.

Fourth, a prototype of a commercial aspiration-type ion mobility spectrometer was mounted in front of a commercial triple quadrupole mass spectrometer. The set-up, which is simple, easy to install, and requires no major modifications to the MS, provides the possibility of gathering fundamental information about aspiration mobility spectrometry.
**Abbreviations**

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AIMS</td>
<td>Aspiration-type ion mobility spectrometry</td>
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<td>APCI</td>
<td>Atmospheric pressure chemical ionization</td>
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<td>API</td>
<td>Atmospheric pressure ionization</td>
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<td>AP-MALDI</td>
<td>Atmospheric pressure matrix assisted laser desorption/ionization</td>
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<td>APPI</td>
<td>Atmospheric pressure photo ionization</td>
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<td>CD</td>
<td>Corona discharge</td>
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<td>CD-APCI</td>
<td>Corona discharge atmospheric pressure chemical ionization</td>
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<td>CID</td>
<td>Collision-induced dissociation</td>
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<td>CV</td>
<td>Compensation voltage</td>
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<td>DC</td>
<td>Direct current</td>
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<td>DIOS</td>
<td>Desorption/ionization on silicon</td>
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<td>DMS</td>
<td>Differential mobility spectrometry</td>
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<td>FAIMS</td>
<td>High-field asymmetric waveform ion mobility spectrometry</td>
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<td>GC</td>
<td>Gas chromatography</td>
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<td>IE</td>
<td>Ionization energy</td>
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<td>IMS</td>
<td>Ion mobility spectrometry</td>
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<td>IMS-FP</td>
<td>Ion mobility spectrometer with Faraday plate</td>
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<td>IMS-MS</td>
<td>Ion mobility spectrometer - mass spectrometer</td>
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<tr>
<td>LC</td>
<td>Liquid chromatography</td>
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<tr>
<td>LDI</td>
<td>Laser desorption/ionization</td>
</tr>
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<td>LOD</td>
<td>Limit of detection</td>
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<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption/ionization</td>
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<td>MS</td>
<td>Mass spectrometry</td>
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<td>PA</td>
<td>Proton affinity</td>
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<tr>
<td>R-APCI</td>
<td>Radioactive atmospheric pressure chemical ionization</td>
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<td>RF</td>
<td>Radio frequency</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>SALDI</td>
<td>Surface-assisted laser desorption/ionization</td>
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<td>SELDI</td>
<td>Surface enhanced desorption/ionization</td>
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<tr>
<td>TAAH</td>
<td>Tetraalkylammonium halides</td>
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<tr>
<td>TIC</td>
<td>Total ion chromatogram</td>
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<td>TNT</td>
<td>Trinitrotoluene</td>
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<td>TWIMS</td>
<td>Traveling wave ion mobility spectrometry</td>
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<tr>
<td>UV</td>
<td>Ultra violet</td>
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List of original papers

This thesis is based on the following studies, which are referred in the thesis by their Roman numbers [I-V]:


Author's contributions

Paper I Main responsibility in modeling of ion optic and software development. Shared main responsibility in designing of IMS. Shared responsibility in experimental research and article writing.

Paper II Shared main responsibility of improvements made to the IMS (heating of the instrument and software improvement).

Paper III Shared main responsibility in designing of ion source platform and IMS, writing article. Shared main responsibility in experimental research and software development.

Paper IV Main responsibility for ion source design, measurements, software development, treating data and writing article.

Paper V Main responsibility for design, carrying out measurements, software development, treating data and writing article.
1. Introduction

Ion mobility spectrometry (IMS) is a measurement technique where mobility separation of ionized analytes occurs by electric field in flow of neutral gas or air under ambient pressure and room temperature [1-5]. The advantages of IMS, including compactness and portability of instrumentation, short separation time (milliseconds scale), and low detection limits (ppt – ppb range [6]), allow a wide range of applications [7, 8].

Although the IMS principle and technique is relatively old, IMS devices have been extensively developed over the last 20 to 30 years [9-12]. A drift tube IMS instrument is traditionally used for IMS separations [1, 9]. However, a number of other instruments such as aspiration-type ion mobility spectrometers (AIMS) [13] and high-field asymmetric waveform ion mobility spectrometers (FAIMS), also called differential mobility spectrometers (DMS) [9, 14], have been successfully developed. Combining with another measurement technique, such as mass spectrometry (MS), increases the selectivity of IMS. The IMS-MS combination has garnered much interest during recent years [3, 10, 12].

Separation by IMS instruments occurs typically at ambient pressure conditions, which makes IMS compatible with many atmospheric pressure ionization techniques, such as electrospray ionization (ESI) [5, 15-20], corona discharge atmospheric pressure chemical ionization (CD-APCI) [5, 21, 22], radioactive atmospheric pressure chemical ionization (R-APCI) [5, 23], and atmospheric pressure photo ionization (APPI) [5, 24]. Various laser based ionization methods have also been used, for example laser desorption/ionization [25-34], laser desorption followed by another ionization method [35-37], matrix-assisted laser desorption/ionization (MALDI) [32, 38-51], MALDI combined with imaging [52-54], and surface-assisted laser desorption/ionization SALDI [55-58]. R-APCI is the most common ionization method, because it does not require external power for operation and therefore is very suitable for instruments built for field use. The use of radioactive material causes some troublesome safety issues however, thus alternative ionization methods are actively built and studied.

The research in this study is focused on the development of instrumentation for atmospheric pressure ionization ion mobility spectrometry and ion mobility spectrometry – mass spectrometry. A drift tube ion mobility spectrometer equipped with a commercial electrospray ionization ion source was designed and built to be mounted to a commercial triple quadrupole mass spectrometer (I). The instrumentation was subsequently utilized for finding possible chemical standards for ion mobility spectrometry – mass spectrometry (II). The same drift tube configuration was also utilized in building a standalone high resolution ion mobility spectrometer – Faraday plate instrument equipped with a multi-mode ion source (III). The multi-mode ion source can also be used in the IMS-MS instrument. A simple ion source set-up for laser desorption ionization, especially for desorption/ionization on silicon (DIOS), to be used with both of the drift tube IMS instruments, was built and tested (IV). A different type of ion mobility instrument, namely a commercial aspiration-type ion mobility spectrometer (IMCell™) was successfully coupled to a commercial triple quadrupole mass spectrometer for characterization studies (V).
2. Aims of the study

The general aim of this study is the development of atmospheric pressure ion mobility spectrometry and ion mobility spectrometry – mass spectrometry instrumentation, including ionization techniques.

The aims of the studies were:

- Development of a high resolution drift tube ion mobility spectrometer for a commercial mass spectrometer to be used for IMS-MS studies (Papers I and II)
- Development of a stand-alone high resolution drift tube ion mobility spectrometer with a multi mode atmospheric pressure ionization source (Paper III)
- Development of simple desorption/ionization on silicone ion source for the ion mobility spectrometer and the ion mobility spectrometer – mass spectrometer (Paper IV)
- Coupling of a commercial aspiration type ion mobility spectrometer (IMCell™) with a mass spectrometer and its characterization (Paper V)
3. Ion mobility spectrometry

Completed in the late 1890s the first investigations of ion mobility in gas-phase under electric field and ambient pressure led to the evolution of mobility theory prior to 1910 [59-62]. Despite the well developed theory, the first analytical IMS instruments become available commercially in 1970 [63]. The IMS technique was initially named “plasma chromatography”, because IMS as an ion-separation technique provides drift time separation, which was considered to be analogous to the retention time separation obtained by chromatographic methods [64-66]. In addition, the IMS technique has also been called “ion chromatography” or “gaseous electrophoresis” [9].

The early IMS instrumentation developed in the 1970s provided no portability and was developed for laboratory use only. At that time, there was no guarantee that the IMS technique would appear as a portable device for in-field use [9]. Technology has improved since that time however, and modern IMS devices have become portable [23, 67-69]. This improved portability has dramatically extended the application ranges of the IMS instruments, which have become widely used analytical techniques not only in the laboratory, but in the field as well.

A traditional IMS instrument is the drift tube IMS, which is based on the determination of velocities of traveling ions in gas-phase under the influence of a weak electric field (<1,000 V cm$^{-1}$) at ambient conditions. The drift tube IMS device typically has a linear drift tube with electric field gradient and countercurrent flow of neutral gas, called the drift gas [1-5, 9].

Due to the demand for further miniaturization of IMS, several new mobility separation techniques have been developed. One of the developed tools is the aspiration-type ion mobility spectrometer (AIMS) [71]. It was developed in 1975 by modification of ion-counting technology for mobility measurements and had a cylindrical design [71], which had three cylindrical electrodes: one central and two outer. The device was used for measurements of mobilities of atmospheric ions. However, most modern aspiration-type spectrometers have been built in planar form [13, 72-74]. The aspiration-type ion mobility spectrometer has a simple design, which is easy to manufacture and is a portable handheld instrument.

Another ion mobility measurement method separates ions based on their mobility dependence on high electric field strength. Commercially available instruments utilizing this principle have been developed, namely differential mobility spectrometers (DMS) [75]. The name high-field asymmetric waveform ion mobility spectrometry (FAIMS) [14, 76] is also used. In contrast to drift tube IMS, where ions are characterized by mobilities, the DMS or FAIMS separates ions based on dependence of ion mobility on strength of electric field [5, 14, 76, 77]. An important advantage of DMA and FAIMS is the possibility for separation of negative and positive ions simultaneously. All of the different atmospheric pressure ionization (API) methods used for the drift tube IMS instruments are applicable to the DMS and FAIMS instruments as well. In addition, an advantage of DMS, FAIMS, and AIMS, in comparison to drift tube IMS, is simplification of the analyzer construction, due to the elimination of ion shutters which are the most difficult component to produce.

The initial step of every mobility separation is ionization of sample molecules. The most common and historical ionization method in IMS is R-APCI [5, 9]. However, ionization in an R-APCI source is initiated by radioactive material which carries a safety issue and requires certificates and permissions to use according to the local
safety regulations. Thus, a number of non-radioactive atmospheric pressure ionization techniques, which are widely used with mass spectrometry, have successfully been combined with IMS instrumentation. The most common alternative ionization techniques combined with IMS devices are corona discharge atmospheric pressure chemical ionization (CD-APCI) [5, 21, 22], atmospheric pressure photo ionization (APPI) [5, 24], electrospray ionization (ESI) [5, 15-20], laser desorption/ionization (LDI) [25-34, 66], and matrix-assisted laser desorption/ionization (MALDI) [32, 38-51].

Although the combination of ion mobility spectrometry with another analysis method can sacrifice portability and high analysis speed of the method, it is often combined with methods like gas chromatography (GC) [63, 78-80], liquid chromatography (LC) [19, 81-83], and mass spectrometry [10, 12, 50, 63, 84, 85]. The combined instruments GC-IMS, LC-IMS, and IMS-MS provide clearly better selectivity for the measurements and therefore more reliable identification of unknowns in the samples can be obtained. The IMS-MS combination in particular, IMS-MS, has gained a lot of attention lately and commercial mass spectrometers with IMS capability are also available [80, 86, 87].

The IMS instrumentation has wide range of application, such as: chemical-weapons monitoring (including handheld and bench-top devices) [88, 89], detection of explosives [8, 90-93], environmental analysis [94-102], medical diagnostics [103-106], bio and proteomics analysis [75, 85, 107], biological and clinical analysis [108-111], food quality analysis [11, 112-118], process control [119-123], illegal drugs detection [92, 124], air quality analysis [78, 125], and fermentation control applications [126]. The list of applications continues to be dramatically expanded. Furthermore, possible future IMS applications cannot be predicted.

3.1. Drift tube ion mobility spectrometry (IMS)

A schematic diagram of a typical drift tube IMS is presented in Figure 1. The main parts of the device are an ion source, ion shutter, drift region, and a detector. In addition, an IMS instrument contains electronics and software to run the instrument.

![Figure 1. Schematic diagram of drift tube IMS. Adapted from [5].](image-url)
Operation of an IMS instrument typically includes the following sequence [1, 4, 5, 9]: gaseous or vaporized sample molecules are conducted into the ion source, where the sample molecules are ionized. An opening of the ion shutter grid (or ion gate) injects a portion of the ionized molecules (ion swarm) into the drift region. The shutter grid opening time is short, typically 50 - 500 μs. This allows formation of compact ion swarm, which is pushed by electric field gradient through the drift region in the direction of the detector. In the drift region, the moving ions undergo collisions with molecules of countercurrent flow of neutral drift gas. Collisions of the ionized analyte molecules with the drift gas molecules produce thermalized ions and the drift velocity \(v_d\) of the ion swarm in the drift region is constant and proportional to the electric field strength \(E\) [1, 5]:

\[
v_d = KE
\]  

In Equation 1, the proportional coefficient \(K\) is the mobility coefficient of the ion swarm in units of \(\text{cm}^2 \text{V}^{-1} \text{s}^{-1}\). The distance between the ion shutter and the detector is the drift length \(d\) [cm, Figure 1]. The time that it takes for the ion swarm to travel through called drift length, is the drift time \(t_d\) [ms]. The velocity of the ion swarm can be calculated based on the drift time, which is measured during an ion mobility separation experiment, and the known drift length (Equation 2).

\[
v_d = \frac{d}{t_d}
\]  

By combining Equations 1 and 2 the mobility coefficient of the ion swarm can be calculated by the following equation:

\[
K = \frac{d}{t_d E}
\]  

Since, the velocities of ions, and therefore their drift times also, are characteristic under an electric field, different mobility coefficients are obtained for the measured ions (Equation 3).

Typically reduced mobility \((K_0)\) coefficients are used (Equation 4), which are obtained by normalization of the mobility coefficients \((K)\) using a temperature of 273 K and pressure of 760 Torr [1]:

\[
K_0 = K \frac{273P}{760T}
\]  

where \(P\) (pressure, Torr) and \(T\) (temperature, K) are properties of the drift gas.

In order to obtain some idea of the magnitude of the parameters, the following example values are presented taken from the book of G.A. Eiceman and Z. Karpas [5]. They report calculated mobility coefficients between 0.8 and 2.4 \(\text{cm}^2 \text{V}^{-1} \text{s}^{-1}\) for ions in the mass range of 14 to 500 u with velocities of 1 to 10 m/s under electric fields of 150 to 300 V/cm [5]. Estimation was done at temperatures of 25 to 250 °C and at ambient pressure. The typical drift times of a modern drift tube IMS are at
millisecond time scale.

The most common method to detect the ions after the flight through the drift region is by a Faraday plate detector. In the detector, arriving ions are neutralized due to the collisions to the collecting electrode of the detector. This process produces an electric current, which is typically in the range of picoampers (pA) [5]. Since the current is low for direct registration, it is amplified and converted to a voltage, which is subsequently digitized and recorded by a computer. A mobility spectrum is obtained as a result of an IMS measurement, which shows the intensity of separated ions (voltage recorded) as a function of the measured drift times. Another common method to present the results is to convert the drift time axis to the reduced mobility scale.

Separation performance of drift tube IMS is characterized by resolving power. A resolving power (R) of a single peak separated in the drift tube IMS is defined by Equation 5 [127]:

\[ R = \frac{t}{\delta t} \]  

where \( t \) is the ion drift time and \( \delta t \) is the ion pulse width at the detector measured at half height of the peak maximum. Equation 5 may be transformed by simple rearrangements for ion mobility scale, Equation 6:

\[ R = \frac{K}{\delta K} \]  

where \( K \) is the mobility of the ion and \( \delta K \) is the mobility range width corresponding to the analytical signal peak at half height of the peak maximum.

The resolving power of commercially available drift tube IMS instruments is in the range of 20 and 60 [6]. However, there are a number of methods to increase the resolving power, such as increasing the strength of electric field in the drift region, decreasing the time of ion shutter grid opening, lowering the temperature of the drift tube, increasing ion charges, increasing electric field homogeneity in the drift region, decreasing Coulombic repulsion, and shortening the collection and amplification rise time [127, 128]. Very good resolving powers have been reported previously. A mobility resolving power of 172 has been reported for a laser desorption IMS-MS instrument with a 63-cm-long drift tube operated under 500 Torr pressure of helium and a drift voltage of 10 kV [129]. Whereas, a resolving power up to 200 has been reported for another ESI-IMS-MS instrument [128], in which high resolution can be obtained due to increased homogeneity of the electric field in the drift tube and shortening of the ion detection time. This instrument had a 13-cm-long drift tube, 280 V/cm drift field was used and it was operated under 700 Torr pressure and at 250 °C temperature [128]. A good resolution, about 155, has also been reported with an ESI-IMS-MS instrument equipped with a drift tube operated with 12,500 voltage and a gate opening time of 0.05 ms [130].

Another ion mobility separation method, which can be considered to be technically close to a drift tube, namely traveling wave ion mobility spectrometer (TWIMS) has been developed recently [87, 131]. The TWIMS is an integral part of an IMS-MS instrument and has a similar design as the drift region in a drift tube IMS instrument, except that the ion gates do not exist. Mobility separation is performed
under reduced pressure (1 mbar) by traveling waves (up to 25 V with velocities in the range of 300–600 m/s), which are formed by a repeated pulse pattern applied on six pairs of electrodes at a time in an 18.5 cm – long drift tube of 61 electrode pairs. [131]. Based on the current literature this commercial IMS-MS device has become very popular in many application areas [80, 87, 132-136].

3.2. Aspiration ion mobility spectrometry (AIMS)

The aspiration ion mobility spectrometer (AIMS) has a simple and miniaturized design [13, 71-74]. Figure 2 shows a schematic figure of a typical modern aspiration-type ion mobility spectrometer, which has a planar design [13, 89, 118, 137]. During the operation of an AIMS instrument, a continuous laminar flow of sample gas is passed between the two parallel plates. First the sample gas encounters an ionization region, where it is typically ionized using a radioactive atmospheric pressure ionization (R-APCI) ion source. The radioactive material used is $^{241}$Am [73, 89, 126] or $^{63}$Ni [74]. Next, the ionized sample molecules are conducted into a drift region, which has a number of deflecting and collecting electrodes on opposite sides of the separation channel. The pairs of deflecting and collecting electrodes are used to produce an electric field perpendicular to the gas flow. Ions traveling in the laminar gas flow are pushed by the electric field towards the collecting electrodes. Ions with high mobility travel across the gas flow faster than the ions with low mobility, and therefore the higher mobility ions travel a shorter distance in the drift region than the ions with lower mobility before reaching a collection electrode. Ions are neutralized at the collecting electrodes, generate an electric current and are registered after amplification. As a result, a mobility histogram is formed consisting of ion intensity versus collecting electrode channel number. The mobility histogram contains analytical information and can be used for identification of analytes [89, 93, 101]. The resolving power of an AIMS instrument is low due to diffusion broadening of ions and the geometry and the number of the collecting electrodes [5, 74]. Separation efficiency can be increased by scanning the electric field [73] or increasing the number of collecting electrodes and variation of the electrodes shapes [5]. However, the AIMS instrumentation has a number of advantages such as: simple design, high portability, and long continuous measurements.

![Figure 2. A schematic diagram of aspiration-type IMS. Adapted from [5].](image-url)
3.3. **Field dependant ion mobility spectrometry (FAIMS/DMS)**

Mobility of ions starts to be dependant on electric field strength under high electric fields [5, 12, 14, 76, 77, 138, 139]. For the IMS instruments (drift tube IMS and AIMS) described in the previous chapters, the electric field strength (E) is kept low and mobility of ions (K) is independent of the electric field (K ≠ f(E/N), where N is number of density of neutral gas) [14, 77]. K is independent of electric field, since the ion energy gained due to the electric field dissipates in collisions with neutral gas molecules when the E/N ratio is below 60 Td (1Td = 10^{-17} V cm^2). However, when the E/N ratio is approximately 60 - 100 Td the electric field strength typically starts to have a significant effect on the mobilities [5, 77].

![Figure 3. Schematic diagram of DMS. Adapted from [5].](image)

High-field asymmetric or differential mobility spectrometers (FAIMS or DMS, here and after only the name DMS is used) have two main designs: planar and cylindrical [5, 12, 14, 76, 77, 138, 139]. Figure 3 shows a schematic picture of a planar DMS device. Two planar electrodes are placed in parallel with a small gap (for example 0.5 mm) between them. As in the AIMS device, the sample is first ionized, often with R-APCI, and then the ions are carried by a gas flow into the DMS separation region. In the separation channel there is an electric field perpendicular to the gas flow. The electric field is generated by the two electrodes, one of which is grounded and into the other RF electric potential is applied. Figure 4 shows an example of the theoretical RF potential profile applied on the other electrode of a DMS device. The RF potential applied typically has an asymmetric rectangular profile (typically with frequency in the range of 0.1 and 1.5 MHz [5, 9, 70, 77]), producing high electric field conditions (typically from 10,000 to 30,000 V/cm [5, 70, 77]) when the maximum voltage is applied and low electric field conditions (e.g. 1000 V/cm [5]) when the low voltage is applied. Since the electric field is perpendicular to the direction of the gas flow, thus the direction of the ion movement, and has an RF cycle, the analyte ions oscillate between the two electrodes. During one RF cycle the ions move following: (i) at the high field conditions (t_{high}, Figure 4) the ions move due to the field towards the grounded electrode and the mobility K(E_{high}) is dependent on the electric field and (ii) at low field conditions (t_{low}, Figure 4), the ions move to the opposite direction...
and $K(E_{\text{low}})$ is field independent. Thus, only the ions with certain differential mobility ($\Delta K = K(E_{\text{high}}) - K(E_{\text{low}})$) are transferred to the detector along with the gas flow. Other ions are neutralized on the electrodes.

![Figure 4. An example of DMS waveform profile. Adapted from [14].](image)

The ions which will be transferred through the DMS device are selected by adjusting the $\Delta K$ value by superimposing a low direct current (DC) voltage, compensation voltage (CV), to the RF potential [14, 138, 140]. A mobility spectrum can be obtained by scanning the compensating voltage in a selected range (e.g. from -12 to 0 V [140]).

DMS instruments have shown good separation efficiency and high sensitivity (ng/L) [138, 139]. The resolving power of DMS devices is typically in the range of 20 to 50 [141]. However, a resolving power of up to 200 was recently reported for peptide mass peaks with 3+ and 4+ charge states [142]. High resolution was obtained by utilization of very high electric fields (up to 29 kV/cm) and using of nitrogen/helium mixture as a supporting gas. Compared to drift tube IMS instruments, the instrumental design of the DMS devices is simple, since it contains no parts like ion gates which can be difficult to produce and are sensitive to vibrations. The simplicity allows miniaturization and high portability of the DMS devices [4, 125, 143]. These instruments have also been successfully combined with mass spectrometry [12, 18, 82, 142, 144-149].

### 3.4. Ion mobility spectrometry – mass spectrometry (IMS-MS)

Although the stand-alone IMS analyzers have many benefits, such as fast analysis time, high sensitivity, simplicity, and portability, their capability to identify unknown analytes is limited [3, 5, 10]. This is due to the fact that the IMS analyzers separate ions based on their structure/cross-section, which does not provide complete information of the identity of the studied sample molecules. Due to this, combining ion mobility spectrometry together with mass spectrometry will provide a very promising hyphenated technique, especially since mass spectrometry characterizes the ions by mass to charge ratio and therefore allows identification of the unknowns [3, 10, 150]. On the other hand, the IMS method supplements the capabilities of mass spectrometry, for example by providing separation of isobaric or isomeric analytes [5, 9, 10, 141], which can be difficult with mass spectrometry alone. In addition, removing the chemical background by IMS before the mass spectrometric analysis enhances the mass spectrometric analysis [9]. For example, DMS has been utilized as a mobility filter before the MS analyzer to reduce chemical
noise and increase signal-to-noise ratio in the analysis of complex samples [140, 144]. Comparing IMS-MS to the hyphenated technique, such as GC-IMS and LC-IMS, reveals that the analysis time obtainable with IMS-MS can be faster than with these techniques. The separation time of gas chromatography or liquid chromatography is in the minutes time scale, whereas with IMS it is at milliseconds time scale which can be followed by mass spectrometry.

The very first IMS-MS instrument was a drift tube IMS (named a plasma chromatograph) interfaced with a quadrupole mass spectrometer [151]. This instrument was also commercially available. Since then, many different mass analyzers have been coupled with various types of IMS instruments, for example a drift tube IMS has been combined with a time-of-flight mass spectrometer [80, 83, 136, 152-155], a quadrupole mass spectrometer [11, 12, 22, 128, 129, 150], an ion trap mass spectrometer [146, 150, 156] and Fourier transform ion cyclotron resonance mass spectrometer [157]. The DMS device has been combined with a quadrupole mass spectrometer [140, 144, 145, 147], with a time-of-flight spectrometer [149], and an ion trap mass spectrometer [142]. The traveling-wave IMS has so far been combined with only a time-of-flight mass spectrometer [131]. It is worth discussing some examples in more detail concerning the combination of a drift tube ion mobility spectrometer and a mass spectrometer, since this type of instrumentation is one of the main topics of this thesis. Combination of AIMS-MS is discussed in more detail in Chapters 5.2.6 and 6.3.

A schematic picture of the first IMS-MS instrument is presented in Figure 5 [63, 158]. This drift tube IMS had two ion gates, was equipped with an R-APCI ion source ($^{63}$Ni) and was interfaced to a single quadrupole mass spectrometer. Three different operation modes for the instrument were reported [158]. Mobility spectra could be collected either in the mass-selected mobility mode and total ion mobility mode [158]. In the former case the mass spectrometer was used to measure selected ions and in the later case it was used to monitor total ion current. In addition to mobility measurements, it could be used for the measurement of mass spectra of the ions formed in the ion source. Gas chromatography could be used for sample introduction.

![Figure 5. A schematic diagram of the Alpha II PC/MS system [158].](image-url)
Figure 6 depicts an instrument which presents the current state-of-the-art of GC-IMS-MS instrumentation [80, 153]. The instrument consists of a secondary electrospray ionization ion source connected to a 10-cm-long desolvation cell, which in turn is mounted in front of a 20-cm-long drift tube with a 38 mm inner diameter. The drift tube is operated under ambient pressure conditions and can be heated. After the drift tube, ions are injected using orthogonal ion injection to a reflectron time-of-flight mass analyzer. Time-of-flight mass spectrometry is very suitable for IMS combinations, because during one IMS duty cycle (milliseconds) the time-of-flight MS can record thousands of mass spectra. As a result, a two-dimensional matrix, which contains both mass and mobility data, can be recorded [10]. However, with the GC-IMS-MS instrument, three dimensional data can be obtained [80]. For example, Crawford et. al. demonstrated three dimensional data for a lavender oil sample analyzed with the GC-IMS-IMS instrument, showing gas chromatographic retention data in addition to the mobility separation data and mass-to-charge data [80]. The instrument presented here, and the general combination of chromatography-IMS-MS, provides an excellent means for the analysis of very complex samples.

Tandem ion mobility configurations combined with a mass spectrometer have also been introduced recently. For example, a DMS device has been combined in a tandem arrangement with a drift tube IMS instrument [12]. The drift tube IMS-DMS set-up was combined with a quadrupole ion trap mass spectrometer and two-dimensional mobility separation was obtained. The second order of mobility separation has also been demonstrated by combination two drift tube IMS instruments in-line with a time-of-flight mass analyzer [154, 155]. This IMS-IMS-MS
instrument also has a collision-induced dissociation (CID) cell between the drift tubes, which allows fragmentation of mobility selected ions and therefore structural analysis of complex peptide mixtures is also possible [155].

4. Ionization methods

4.1. Electrospray ionization (ESI)

Electrospray ionization (ESI) is an atmospheric pressure ionization method, which is widely used in mass spectrometry [159]. Electrospray ionization is well suitable for ionic, moderately polar and polar molecules. First suggested by Dole et al. in 1968 for gas phase ion production [160], ESI was successfully applied for the analysis of large bimolecules in 1989 [161]. Since 1989 ESI as an ion ionization method has been studied in detail and a very large number of ESI applications have been developed.

![Figure 7. Schematics of the ESI ionization process. Adapted from [159].](image)

Perhaps the most important property of ESI is that it allows ionization of analytes directly from liquid phase. In electrospray ionization, an analyte solution flows through typically a stainless steel spray needle, which has a high electric potential (around 2 – 4 kV) relative to a counter electrode (Figure 7). Due to the high electric field and liquid flow, a spray of charged droplets (the same polarity as the needle) is formed at the tip of the electrospray needle, which moves towards the counter electrode. While the charged droplets are flying to the counter electrode, solvent evaporation occurs and the size of the droplets decreases to a size in which the surface tension of the droplets cannot hold the charge anymore and droplets break down to smaller charged droplets. This droplet breaking process is repeated until charged analyte molecules are produced [162]. A nebulizer gas flow co-axial with the liquid sample flow is often used in an ESI ion source to assist the spray formation [159].

An important advantage of ESI is that it is a soft ionization method, which can produce positive or negative ions. Due to the softness of the ionization, very large biomolecules can also be measured, for example up to 130 000 Da was reported.
already in the 1980s [161]. The ESI ionization process produces protonated molecules ([M+H]+) in the positive ion mode, while deprotonated ions ([M-H]-) are formed in the negative ion mode. Adduct formation is also often possible in both positive and negative ionization modes (for example, formation of [M+Na]+, [M+HN4]+, or [M+Cl]- ions) [162]. Multiple charged ions can also be formed, for example for proteins which contain many protonation sites [163].

Electrospray ionization has been successfully used with wide range of IMS devices and applications [5, 15-20, 164-166]. Introduction of liquid phase samples into IMS instruments widens the applicability of IMS, but direct introduction of liquid samples can also be disadvantageous. If the IMS instrument is not heated to a temperature above the boiling point of the sample solvent it can condensate on the drift tube walls, which causes contamination and a long lasting memory effect [5]. A heating of the drift tube to a temperature above the boiling point of the ESI solvent has shown to prevent condensation and reduce the memory effects.

In principle, the mounting of an ESI ion source to an IMS instrument is simple. One difficulty could be application of a high enough voltage to the ESI needle since relatively high voltages are already used in the drift tubes and the ESI needle is required to be at a few kV higher potential. An example of the use of ESI with IMS is presented in Figure 8 [165]. This ESI-IMS instrument has an electrospray needle, which is attached to a drift tube and insulated from possible contaminations from ambient laboratory air. A focusing screen is installed at entrance to the desolvation region to improve ion movement to the desolvation region. The ESI needle has a water/air cooling jacket to prevent signal instability, which can occur if solvent starts to evaporate inside the ESI needle before it is sprayed, due to the heating effect of the heated drift gas [165, 166].
4.2. Radioactive atmospheric pressure chemical ionization (R-APCI)

The most common and historically the most used ionization method in IMS instrumentation is radioactive atmospheric pressure chemical ionization (R-APCI) with $^{63}$Ni [5, 63]. Formation of the positive analyte ions starts with emission of high energy electrons from $^{63}$Ni [9]. Next, the high energy electrons collide with atmospheric molecules (purified air or nitrogen) generating, for example, N$_2$ ions and then primary reactant ions react with water molecules to form (H$_2$O)$_n$H$^+$ reactant ions. The number “n” depends on moisture level and temperature of the gas in the ion source [9]. Formation of other reactant ions is also possible, such as NH$_4^+$ and NO$^+$, depending on the impurities of the sample gas. Analyte molecules are ionized by proton transfer reactions if their proton affinity is larger than that of neutral water cluster, as a product [M+H]$^+$ ions are formed. Production of dimers [2M+H]$^+$ and cluster ions is also possible.

Ionization of oxygen molecules by attachment of thermalized electrons leads to the formation of negative reactant ion (H$_2$O)$_n$O$_2^−$. Further reactions of the reactant...
ions with the analyte molecules produce negative product ions, [M-H]−, by proton transfer reactions.

As an alternative to the 63Ni radioactive material, the use of Tritium (T), which has lower radiation hazard, has been reported [167]. Tritium emits electrons having maximum energy of 18 keV and mean energy of 6.5 keV. The half-life time of the tritium is significantly shorter than that of 63Ni, approximately 12 years. Another radioactive material commonly used in R-APCI is 241Am [9], which emits alpha particles with an energy of 5.4 MeV. Formation of the analyte ions starts with collisions of the emitted alpha particles with air molecules forming primary ions, which subsequently react with analyte molecules to form analyte ions [9]. Due to the short effective range of the alpha particles, R-APCI sources with 241Am radioactive material are suitable for devices with small ionization region dimensions, for example it is used in AIMS devices [13, 72, 89, 93, 101].

The R-APCI is a compact, simple and stable ion source, which requires no external power for operation and no maintenance. Typically, the radioactive material is simply placed inside the ionization or reactant region. Thus, the R-APCI is very good for portable and hand-held devices. However, the utilization of radioactive material requires special permits and licenses in accordance with radioactive safety regulations. An additional safety feature of R-APCI is that the solvents and compounds used with the R-APCI source must be chemically neutral to the radioactive material. The R-APCI is suitable for gaseous and volatile moderately polar and polar compounds. Liquid samples have to be evaporated to the gas phase before ionization.

4.3. Corona discharge atmospheric pressure chemical ionization (CD-APCI)

The corona discharge atmospheric pressure chemical ionization (CD-APCI) is a non-radioactive alternative to R-APCI [9]. Formation of the analyte ions in a CD-APCI is similar to that presented above for R-APCI with 63Ni radioactive source (see Chapter 4.2) [5, 9]. In the CD-APCI source the high energy electrons are generated by a corona discharge instead of the 63Ni. The high energy electrons react with nitrogen in air to form ions such as N2+, which undergo ion-molecule reactions to form analyte ions by reactions analogous to those in the R-APCI source. Like the R-APCI, the CD-APCI is suitable for gaseous or volatile moderately polar and nonpolar compounds. However, due to a lack of radioactive material, the CD-APCI has no limitations concerning safety regulations and is free of radioactive contamination risk. A downside for CD-APCI sources is the need for additional power, however, which decreases the portability of IMS.

Figure 9 shows a typical set-up for CD-APCI drift tube IMS instrumentation [168]. Compared to an R-APCI source the design of a CD-APCI is more complicated since a corona needle and an additional high voltage source is needed. The corona discharge is generated by applying a high voltage (1 – 3 kV) between the corona needle and a target electrode (see Figure 9). The high electric field produces a strong non-uniform field at the tip of the corona needle (~10⁹ Vcm⁻¹ [9]) and therefore corona discharge and high energy electrons are formed.
A limitation of the CD-APCI method is that the applied field on the CD needle has an effect on the available analyte ionization reaction routes [22]. For example, in negative ion mode, formation of analyte ions is disturbed by the presence of cluster ions related to NO$_2$, which have higher electron affinity than O$_2$ [22]. Production of these NO$_2$ related clusters can be significantly reduced by a pulsed CD-APCI set-up, where a pulsed voltage is applied between two the electrodes and preferred formation of (H$_2$O)$_n$O$_2^-$ reactant ions observed compared to NO$_2$ cluster ions [22, 169].

### 4.4. Atmospheric pressure photo ionization (APPI)

Atmospheric pressure photoionization (APPI) is a widely used ionization method in mass spectrometry, which facilitates the ionization of gaseous samples (or evaporated liquid samples) by energetic photons [170-173]. The APPI method is well suited for the ionization of volatile non-polar compounds.

In positive ion APPI, direct photoionization of analyte (M) occurs if its ionization energy (IE) is lower than the photon energy (hν, where h – Planck constant, and ν – frequency of photon) (Equation 7).

$$h

\nu + M \rightarrow M^{+*} + e^- \quad (7)$$

The photon energy in the typically used UV lamps is around 10 eV (8.4 eV xenon, 10.0 and 10.6 eV krypton, and 11.6 eV argon UV lamps [9]) and therefore ionization of many organic compounds is possible since their IE’s are generally between 7 and 10 eV. In order to increase ionization efficiency, and to extend the range of APPI applications, a dopant-assisted photoionization method has been suggested [173]. In the dopant-assisted atmospheric pressure photoionization, an organic solvent which is easily ionized by the photons (dopant) is added into the ionization region. The energetic photons ionize dopant molecules, depending on the dopant, different reactant ions can be formed and finally the analyte molecules are typically ionized by charge exchange (M$^+$ ions formed) or proton transfer ([M+H]$^+$ ion is formed). One of the most common dopant compounds is toluene, which has low IE (8.8 eV [174]) and...
is efficiently ionized by the UV lamps used. The ionization processes in the negative ion APPI and in negative ion APCI are similar. The source of the ionizing electrons is the dopant or metal surfaces in the ionization chamber [175].

The APPI method has been successfully used with IMS instruments [115, 176-178]. The APPI-IMS combinations can technically be considered to be close to the CD-APCI-IMS instruments except that the corona needle is replaced with a photoionization lamp. Of course the dopant assisted APPI needs an additional method for the introduction of the dopant, which can be added in the sample solvent or vaporized into the auxiliary gas. Figure 10 shows a typical APPI set-up used in mass spectrometry, applicable also in ion mobility spectrometry. The UV lamp is positioned in front of a heated nebulizer, which is often used for sample introduction. In the heated nebulizer, a liquid sample is vaporized by heating the device and with a help of a nebulizer and an auxiliary gas with dopant. As for CD-APCI, the APPI method requires an additional power source compared to R-APCI, which makes it less favorable for portable instruments. In addition, the use of dopant complicates the system.

![Figure 10. A schematic diagram of APPI source for mass spectrometry [173].](image)

### 4.5. Desorption/ionization on silicon (DIOS)

Desorption/ionization on silicon (DIOS) [179] is a method related to laser desorption/ionization (LDI) technique [180, 181]. In the LDI, laser irradiation desorbs or vaporizes and at the same time ionizes analyte molecules, which are typically deposited on a surface. Ionization efficiency of LDI was increased by modification the method to matrix-assisted laser desorption/ionization (MALDI) [182]. In the MALDI a matrix compound, which is mixed with the sample, gives enhancement of the ionization efficiency [180, 181]. The matrix is a compound, which has high absorption ability of the laser irradiation. The MALDI is possible not only in vacuum, which is mostly used in mass spectrometry, but also under atmospheric pressure conditions (AP-MALDI) as done in mass spectrometry [183, 184] or in ion mobility spectrometry [42, 45]. Due to more efficient cooling of matrix molecules at atmospheric pressure, the AP-MALDI method is softer and analytes are less fragmented than in vacuum MALDI [183]. In both vacuum MALDI and AP-MALDI
methods, the matrix produces high chemical background in low mass range (up to m/z 700 [185]). In order to avoid the matrix background, surface assisted laser desorption/ionization (SALDI) technique was introduced [185, 186] for mass spectrometry. IMS instruments have also been successfully combined with MALDI and related techniques. For example, IMS have been combined with LDI [25-34], laser desorption followed by another ionization method [35-37], MALDI [5, 32, 38-45, 47-51], and with MALDI imaging [52-54].

The DIOS is a variation of the SALDI method, in which porous silicon (pSi) is used as a surface [179]. A low background at low mass range [179] and the fact that signal persists for a relatively long time in AP-DIOS as demonstrated with MS measurements [187] are important advantages of the method when its combination with atmospheric pressure IMS are considered. A pSi was recently utilized in an ion source of an IMS drift tube instrument for gas phase trinitrotoluene (TNT) detection in negative ion mode [58]. The main role of pSi was to produce electrons by laser irradiation for sample ionization and possibility of DIOS was commented. Observation mobility peak in positive ion mode due to desorption/ionization of molecules adsorbed to the surface was also mentioned. The use of non-porous rough surface for laser desorption/ionization with IMS detection has also been proposed in a patent [188]. Other IMS studies which have been found and which in our opinion can be counted to belong to the SALDI methods are utilization of gold clusters [55], gold nanoparticles [52], or carbon nanomaterial [56] as SALDI materials. In addition, polypyrrole has been used as a coating in solid-phase microextraction and the same polypyrrole coated fiber was used for surface enhanced desorption/ionization (SELDI) [57, 109].

Figure 11 depicts an example of an API-MALDI ion source and drift tube IMS combination [42]. In this API-MALDI-IMS instrument, a target was placed 1-2 mm away from the orifice of the drift tube. Electric potential (+2.7 kV) was applied to the target. Samples mixed with α-cyano-4-hydroxycinnamic acid (CHCA) matrix were ionized by a 337 nm laser. An advantage of this API-MALDI-IMS is that the set-up had no ion gates. Drift times were measured from the laser shot. [42]

![Figure 11. A schematic diagram of API-MALDI-IMS [42].](image-url)
5. Experimental

This chapter describes the materials, chemicals, samples, and instrumentation utilized in the presented work. Detailed information and description can be found in the original Papers I-V.

5.1. Chemicals, materials and equipment

The following items are listed in the tables: products, material and equipment (Table 1), and chemicals (Table 2).

Table 1. List of products, materials and equipment

<table>
<thead>
<tr>
<th>Product / Material</th>
<th>Manufacturer / Supplier</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature control unit, Cal 3000</td>
<td>Cal Controls Ltd., UK</td>
<td>II</td>
</tr>
<tr>
<td>Laser set-up construction elements</td>
<td>Thorlabs, Newton, New Jersey, USA</td>
<td>IV</td>
</tr>
<tr>
<td>Corona needle</td>
<td>NHS Oy, Hyvinkää, Finland</td>
<td>III</td>
</tr>
<tr>
<td>Dew point sensor DMT242</td>
<td>Vaisala, Helsinki, Finland</td>
<td>III</td>
</tr>
<tr>
<td>ES111 laser energy sensor</td>
<td>Thorlabs, Newton, New Jersey, USA</td>
<td>IV</td>
</tr>
<tr>
<td>Fused silica capillary (160 OD x 100 μm ID)</td>
<td>Applied Biosystems – Sciex, Canada</td>
<td>I,II,III</td>
</tr>
<tr>
<td>Heater power supply</td>
<td>Meyer Vastus Oy, Monninkylä, Finland</td>
<td>II</td>
</tr>
<tr>
<td>Heating wire, 0.40 mm (Kanthal D)</td>
<td>Farnell, Helsinki, Finland</td>
<td>II</td>
</tr>
<tr>
<td>LabView software</td>
<td>National Instruments, Austin, TX, USA</td>
<td>I,II,III,V</td>
</tr>
<tr>
<td>Membrane pump MPC201E</td>
<td>ILM-VAC GmbH, Germany</td>
<td>III</td>
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<tr>
<td>nano:ES platform</td>
<td>Thermo Fisher Scientific, Denmark</td>
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<td>NAPLES 355 laser</td>
<td>Passat Ltd, North York, Canada</td>
<td>IV</td>
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<td>Nitrogen generator, N₂-mistral-O</td>
<td>VWR International Oy, Helsinki, Finland</td>
<td>IV</td>
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<tr>
<td>Nitrogen generator, NGLCMS20</td>
<td>Labgas Instruments Co, Espoo, Finland</td>
<td>I – III,V</td>
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<tr>
<td>Porous silicon (pSi)</td>
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<tr>
<td>PPS-1206 power supply</td>
<td>Motech Instruments, Taipei Hsien, Taiwan</td>
<td>V</td>
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<td>Python programming language</td>
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<td>SciPy scientific package for Python</td>
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<td>Simion 7 software</td>
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<tr>
<td>Syringe pump MC 789100C</td>
<td>Cole-Parmer Instrument Co., Vernon Hills, IL, USA</td>
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<td>TSI 4140 mass flow meter</td>
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<tr>
<td>TTL generator PC board</td>
<td>Rudnev-Shiliaev, Moscow, Russia</td>
<td>I</td>
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<tr>
<td>UV lamp</td>
<td>Cathodeon / Heraeus Noblelight, Cambridge, UK</td>
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Table 2. List of chemicals used in the study

<table>
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<th>Chemical</th>
<th>Manufacturer / Supplier</th>
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<td>2,4-dimethylpyridine</td>
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<tr>
<td>2,6-di-tert-butylpyridine</td>
<td>Aldrich, Steinheim, Germany</td>
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<td>Dimethyl methylphosphonate</td>
<td>Fluka, Buchs, SG, Switzerland</td>
<td>V</td>
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<td>Formic acid</td>
<td>Riedel-de Haën, Seelze, Germany</td>
<td>I,II</td>
</tr>
<tr>
<td>Methanol</td>
<td>Baker, Deventer, Holland</td>
<td>I,III</td>
</tr>
<tr>
<td>Methanol</td>
<td>Sigma-Aldrich, Steinheim, Germany</td>
<td>IV</td>
</tr>
<tr>
<td>Ortho-phthalic acid</td>
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<tr>
<td>Pentane</td>
<td>Labscan, Dublin, Ireland</td>
<td>III,V</td>
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<td>Tertaoctylammonium bromide</td>
<td>Aldrich, Steinheim, Germany</td>
<td>II,III</td>
</tr>
<tr>
<td>Tetrabutylammonium iodide</td>
<td>Fluka, Buchs, SG, Switzerland</td>
<td>I,II,IV</td>
</tr>
<tr>
<td>Tetradecylammonium iodide</td>
<td>Fluka, Buchs, SG, Switzerland</td>
<td>II,III</td>
</tr>
<tr>
<td>Tetradodecylammonium chloride</td>
<td>Fluka, Buchs, SG, Switzerland</td>
<td>II,III</td>
</tr>
<tr>
<td>Tetraethylammonium iodide</td>
<td>Sigma-Aldrich, St. Louis, USA</td>
<td>II,III</td>
</tr>
<tr>
<td>Tetraheptylammonium iodide</td>
<td>Fluka, Buchs, SG, Switzerland</td>
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<tr>
<td>Tetrahexylammonium iodide</td>
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<td>Tetrapropylammonium iodide</td>
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5.2. Instrumentation

The instrumentation that was developed and the most important commercial instrumentation is described in this chapter. First, the five types of API sources for IMS and IMS-MS instrumentation used and built in the study are described. Then, the ion mobility spectrometry development of the study is described.

5.2.1. Electrospray ionization (ESI)

The ESI source used in the study (I,II,III) was a commercial IonSpray source (Applied Biosystems – Sciex, Canada). It was used together with a Sciex API-300 and an API-365 mass spectrometer (Applied Biosystems – Sciex, Canada) and with the IMS Faraday plate instrument. Only two modifications were made to the IonSpray source, namely changing the original 8 kV voltage connector to a 20 kV high voltage connector and the use of an external high voltage power supply instead of internal power supply of a mass spectrometer. The voltage on the ESI needle
could be varied from 8 to 15 kV. Figure 12 shows a schematic diagram of the ESI-IMS-MS set-up and Figure 13 shows a photograph of the IonSpray source. A syringe pump was used for introducing liquid samples into the ESI source through a fused silica capillary. Nebulizer gas was supplied by the internal mass flow control of the mass spectrometer (I,II) or from an external mass flow controller (III).

Figure 12. A schematic diagram of drift tube IMS with the ESI ion source set-up (from Paper I).

Figure 13. A picture of the Ion Spray ESI ion source.
5.2.2. Multi-ion source platform (CD-APCI, R-APCI, and APPI)

The multi-ion source platform was developed for the easy use of different atmospheric pressure ionization methods in the IMS-FP and IMS-MS instruments (III). Capability for corona discharge atmospheric pressure ionization (CD-APCI), radioactive atmospheric pressure ionization (R-APCI) and atmospheric pressure photoionization (APPI) was built (III). It is also possible to use ESI with the platform, however ESI capability was not built. Instead a commercial ESI ion source was used (see Chapter 5.2.1).

The most important part of the multi-ion source platform is a triangular stainless steel pyramid installed onto the IMS inlet cup (Figure 14). Figure 14a shows a schematic picture of the pyramid and the entire ion source set-up, whereas Figure 14b shows a detailed picture of the pyramid. The ion source housing is a stainless steel chamber with two windows which allow inspection of the ion source, also during operation of the instrument. Figure 15 shows pictures of hardware for different ionization modes, (a) CD-APCI, (b) R-APCI and (c) APPI installed into the stainless steel pyramid on the IMS-FP device. Switching between different ion source hardware typically takes 10-15 minutes. All the ionization modes of the multi-ion source platform can be operated in both, negative and positive polarity.

In the R-APCI mode, a metal foil with $^{241}$Am (10 MBq) was placed into a custom-made cylindrical stainless steel holder, which was screwed at the top of the truncated pyramid using a Teflon insulator (Figure 15b). The insulation allows the application of potential difference between the holder and the pyramid. The distance between the radioactive foil and IMS orifice was about 26 mm.

In the APPI set-up, a krypton UV lamp with 10 and 10.6 eV proton energies was installed into a lamp holder, which was placed on one face of the triangular stainless steel pyramid through a cylindrical hole (Figure 15c). A custom made power supply is used for the operation of the UV lamp.
The CD-APCI source is assembled by installing a Teflon holder with a corona needle into a hole in the face of the triangular pyramid (Figure 15a). The holder places the needle in front of the drift tube orifice at about 8 mm distance and a 45° angle.
Figure 15. Multi-ion sources platform set-up in (a) CD-APCI, (b) R-APCI, or (c) APPI mode.

Sample introduction into the ion source was done through the hole at the top of the truncated triangular pyramid ("Sample-in", Figure 15). The hole allows, for example, the use of a heated nebulizer or a tube for the introduction of liquid or gaseous samples into the ion source. Gaseous samples were typically prepared by using a gas calibrator method, in which a 1% solution of an analyte in pentane was injected into stream of nitrogen using a syringe pump (III).
5.2.3. Desorption/ionization on silicon (DIOS)

A simple DIOS ion source set-up was designed for IMS-MS and IMS-FP devices (IV). The set-up is similar to the set-up demonstrated by Bramwell et al. [42] (Figure 11). Figure 16 shows schematics of the set-up and a photograph showing the actual ion source set-up on the IMS-MS instrument. The set-up was made utilizing a nano:ES platform, which allows easy use of the ion source set-up on both the IMS-FP and the IMS-MS instruments. A custom made lens system was mounted on the NanoES platform and used for focusing of the laser beam from (a 3rd harmonics of Nd:YAG laser) on the sample surface. A piece of silicon containing a porous silicon (pSi) spot was placed on the custom made sample holder inside the orifice of a drift tube. Samples were added to the pSi spot pieces with a pipette in small aliquots and allowed to dry before mounting of the sample holder to its place.

Figure 16. (a) A schematic diagram of the IMS-MS and IMS-FP instrumentation (b) a photograph of the laser desorption ion source mounted on the IMS-MS instrument (from Paper IV).
5.2.4. Ion mobility spectrometer – mass spectrometer (IMS-MS)

A schematic diagram of the atmospheric pressure ionization ion mobility spectrometer – mass spectrometer (API-IMS-MS) instrument and a photograph of the device are shown in Figure 17. The device has three main parts: an API source, a drift tube IMS, and a triple quadrupole mass spectrometer. The drift tube IMS module designed and constructed at the Moscow Engineering Physics Institute (State University), Russia with cooperation of University of Helsinki (I,II), is built to be compatible with commercial API Sciex 300, 365 and 3000 instruments and can be installed in-between an API source and a mass spectrometer. Only minor modifications are needed to the API source (e.g. see Chapter 5.2.1, ESI modifications needed) and the mass spectrometer (I).

The main parts of the drift tube IMS are the actual drift tube, a chamber surrounding the drift tube, and an electronics module (Figure 18). The drift tube is divided to inlet (i.e. ion source), desolvation, drift, and extraction regions. The inlet region is separated from the desolvation region by a 6 mm orifice. The desolvation region has a length of 76.5 mm and is separated by a Bradbury-Nielson type bipolar ion gate from the 133 mm-long drift region. The drift tube was constructed from 23 stainless steel ring electrodes, two stainless steel extraction electrodes, two bipolar grid ion gates, and 26 Teflon rings. The drift tube diameter is defined by the inside diameter of the ring electrodes, which is 50 mm. The width of the ring electrodes is 8 mm inside the drift tube. The Teflon rings are used for insulation and placed between the ring electrodes. Electric field with linear gradient was formed by connecting a high voltage custom made power supply to the resistive divider, which is assembled from high precision 1 MOhm resistors.

The ion gates were assembled from two sets of parallel Chrome 20 Nickel 80 alloy wires. The wires had a 60 μm diameter and were located 600 μm from each other. The gates can be closed for ions by applying ±50 V to the adjacent wires or can be open if all wires are set to a potential equivalent to the position of the gates in the drift tube.

The electronics module of the IMS instrument includes four positive and four negative high voltage supplies, two ion gate controllers and a TTL timing generator. Three of the four power supplies have a range of 0 to 10 kV and one has a range of 0 to 15 kV (positive and negative side) (I).

A heater assembled to the drift tube was made from 0.40 mm heating wire (Kanthal D). For electric insulation purposes, the wire was installed inside glass tubes which were mounted into the holes in ring electrodes. Power for heating was obtained from an 150 DC voltage heater power supply controlled by a temperature control unit Cal 3000 (II).

The mass spectrometers used for API-IMS-MS were a commercial triple quadrupole API-300 (I,II) mass spectrometer and API-365 mass spectrometer (IV). The IMS drift tube was installed between an API ion source and the mass spectrometer front end. The curtain plate of a mass spectrometer was replaced by a custom made holding plate which permits the drift tube to be mounted to a mass spectrometer. Additionally the ion source support, which contains the exhaust line, was moved by installing two stainless steel metal plates in-between the ion source support and the mass spectrometer body. The curtain gas of the mass spectrometer was used as a drift gas for the drift tube. The API-IMS-MS device can be operated in the following modes: MS only, continuous ion flow, selected mobility monitoring dual-gate, and mobility scan dual-gate (I).
Figure 17. (a) A schematic diagram of the API-IMS-MS instrumentation (from Paper I) and (b) a photograph of the API-IMS-MS device.
Figure 18. (a) A schematic diagram of the drift tube IMS showing also mass spectrometric connection and an ESI ion source (from Paper I) and (b) a photograph of the drift tube.

5.2.5. Ion mobility spectrometer with Faraday plate (IMS-FP)

The IMS-FP was developed as a stand alone device (Figure 19) (III, IV). The IMS-FP consists of a drift tube, a Faraday plate detector and an electronics module (supplied by Moscow Engineering Physics Institute (National Research Nuclear
University), Moscow, Russia). Different atmospheric pressure ionization methods can be used for sample ionization utilizing the multi-ion source platform developed or for example a commercial IonSpray source (see Chapters 5.2.1, 5.2.2, and 5.2.3) can be used. The drift tube has the same design as that described in Chapter 5.2.4, except that the detector part is different. The main difference is that the IMS-FP instrument uses a Faraday plate detector (III) for ion detection instead of a mass spectrometer and therefore the same kind of extraction region is not needed then for the IMS-MS set-up. An additional difference is that heating capability was not built for the IMS-FP instrument. There is a small difference in the drift length between the two instruments, 138.5 mm for the IMS-FP instrument. For controlling the IMS-FP device an advanced version of electronics was used.

![Diagram of IMS-FP instrument](image)

Figure 19. (a) A schematic diagram of IMS-FP instrument (from Paper III), and (b) a photograph of the IMS-FP device.
5.2.6. Aspiration type ion mobility spectrometer – mass spectrometer (AIMS-MS)

A prototype aspiration ion mobility spectrometer (IMCell™) was coupled to a commercial triple quadrupole mass spectrometer API-300 for the collection of fundamental information regarding the operation of the aspiration ion mobility spectrometer (V). The combined instrumentation has the following main parts: an aspiration ion mobility spectrometer, an interface plate, and a mass spectrometer (Figure 20). The ion source in the aspiration IMS device was R-APCI ion source ($^{241}$Am).

Figure 20. A schematic diagram of IMCell™-MS experimental set-up (from Paper V).

In order to mount the aspiration IMS to the mass spectrometer, the curtain plate of the API-300 mass spectrometer was replaced with a custom made interface plate to which the IMCell™ was attached (Figure 21). The interface plate was made from polymeric material and had a 10 mm orifice for gas flow and ion introduction to the mass spectrometer. A relatively large orifice was used to minimize gas flow disturbance. The interface plate held the IMCell™ in a way that the third electrode of the device was in front of the MS orifice. Ions from the ImCell™ entered into the mass spectrometer via a slot (5 x 0.5 mm) made in the third collecting electrode. Gaseous samples were produced as described earlier, by injecting/vaporizing a small flow of analyte solution into a stream of nitrogen (V).
Figure 21. A schematic diagram of IMCell™-MS interface (from Paper V).

Operation of the IMCell™ device can be described by two voltages, $U_{\text{base}}$ and $U_{\text{bias}}$. The $U_{\text{base}}$ is applied to all the collecting electrodes and $U_{\text{bias}}$ is the potential difference between collecting and deflecting electrodes. The $U_{\text{bias}}$ voltage at which an ion with an ion mobility $K$ is collected before it reaches the detection channel at the distance $L$ can be calculated by the following equation (V):

$$U_{\text{bias}} = \frac{h^2 v}{KL}$$

where $v$ is the gas flow velocity, $L$ is distance from the beginning of the collection section and $h$ is the distance between deflecting and collecting electrodes (V).

5.3. Software

The following software was developed for the IMS instrumentation:

- A controlling program for the TTL generator, which allows operation of the IMS-MS instrument in different scanning modes (written in LabView, I,II)
- A data collecting and processing program for the IMS-FP (written in LabView, III)
- A program for mobility calculations and data presentation (written in Python, IV)
- A program for automatic control of IMCell™ voltages (written in LabView, V)
6. Results and discussions

6.1. Development of drift tube for ion mobility spectrometry and ion mobility spectrometry – mass spectrometry instrumentation

6.1.1. Modeling and development of ion optics of the drift tube

The drift tube in the IMS-MS instrument contains four main parts: inlet, desolvation, drift, and extraction regions (Figure 18a) (I). The extraction region is replaced with the Faraday plate detector in the IMS-FP instrument (Figure 19a) (III). The entire drift tube is mounted inside stainless steel housing. Housing of the drift tube protects it, allows mounting the drift tube to a mass spectrometer or allows connection of the Faraday plate detector to drift tube, allows the use of different atmospheric pressure ionization methods, and high-voltage power connectors and heater connectors are positioned in the housing. Installation of the drift tube to the mass spectrometer and installation of most of the ion sources to the housing allow hermetic isolation of the inner region of the drift tube from the outer region of the drift tube (space between the housing and drift tube). The outside region is separately pumped via a flange in the housing. In addition, the drift tube of the IMS-MS instrument is equipped with heater elements. IMS-MS can be heated roughly up to 150 °C, the upper temperature is mainly limited by thermal characteristics of resisters used in the HV divider.

The function of the inlet region is to act as ionization area and allows efficient extraction of the ions from an ionization area into the desolvation region through a 6 mm orifice. In the IMS-FP version of the drift tube, the inlet region was slightly modified compared to the IMS-MS version, but at present the IMS-MS instrument also has the same kind of inlet cup as in the IMS-FP (see Figure 18 and Figure 19). The desolvation region (length 7.5 cm) is designed for evaporation of the remaining solvent from solvated ions in the counter flow of the drift gas. Bare ions enter into the 13.3-cm-long drift region through the entrance ion gate, which separates the drift region from the desolvation region. In the drift region, an ion swarm, which is formed by the ion gates, undergoes mobility separation. Another ion gate at the end of the drift region allows selection of ions with certain mobility and injecting the ions into the 3.05-cm-long extraction region. The extraction region focuses the ions via an orifice plate into the mass spectrometer. In contrast, the IMS-FP instrument’s second gate is typically not used and the extraction region is replaced with a Faraday plate detector (III).

One of the major efforts in designing the drift tube was modeling the electric fields in the drift tube, which allowed optimization of the design (I). The main design goals were to minimize ion losses and to obtain a drift tube with good resolving power. The modeling was done using SIMION 7 software, which provides simulation of electric fields and also the possibility to include a hard-sphere collision model in the simulation.

At first, simulation of an electric field in desolvation and drift regions was performed in order to define optimal shape and positions of the ring electrodes, which form the electric field inside the drift tube. The optimal ring electrode has a 50 mm inner diameter (i.d.), a T-shape cross section with 8 mm electrode width inside the drift tube, thin walls and an outer edge with a 100 mm diameter. The electrode...
shape and positioning of the electrodes with a 1 mm gap from each other provides a homogenous electric field with smooth gradient and allows focusing of ions on the axis of the drift tube. The homogeneity of the electric field is especially important for obtaining good resolving power.

The second important goal of the modeling was to determine optimal operation conditions for the ion gates. Time-widths of ion packets formed by applying on the ion gates different opening voltages (30, 40, and 50 V) at different injecting times (100, 200, and 500 ms) were obtained by integrating a hard-sphere collision model into the SIMION 7 software (I). The simulation showed that the opening voltages had an insignificant effect on the time-width of the formed ion packets. A voltage of 50 V was selected for subsequent work.

The third simulation task was to optimize design of the inlet region for efficient ion extraction from the ionization area into the desolvation region. A number of designs of the inlet region were modeled before a reasonable compromise was obtained. Figure 22a shows simulated electric fields in the inlet region of the ESI-IMS-MS instrument with the electrospray needle (I). The orifice diameter, 6 mm i.d., was also a compromise. The orifice has to be sufficiently small to cause efficient solvent evaporation from the charged droplets by drift gas flow, but at the same time, be large enough to provide good ion extraction due to strong electric field at the orifice (I).

Figure 22. (a) A SIMION 7 model used to determine the electric fields within the inlet region of the drift tube by electrostatic calculations (from Paper I), (b) a SIMION 7 model of the extraction region of the drift tube.
The fourth simulation task was to study ion focusing in the extraction region of the drift tube. This work helped to find the best conditions to focus ions into the MS orifice. The extraction region is defined by the exit ion gate, two coaxial cylindrical electrodes and the MS orifice (pinhole) plate. Figure 22b shows a modeling example of the extraction region. The ion trajectories start from the exit ion gate and the simulation show how the applied electric field on the extraction region causes ion focusing. The electric field shape in the extraction region can only be varied by changing the voltage on the exit ion gate, since the voltage on the coaxial cylindrical electrodes is defined by the mass spectrometer. Based on the modeling, the optimal exit ion gate voltage was 1500 V.

6.1.2. Performance characteristics of the drift tubes

The performance characteristics of the drift tube were mostly determined using the ESI-IMS-MS instrument, but characterization studies were also done using the IMS-FP instrument. It is expected that both drift tubes have similar characteristics due to similarity in the design.

Ion transmission efficiency of the IMS-MS was estimated by comparison of data obtained in the MS only mode and in the continuous ion flow mode. This was done in order to obtain information about possible ion losses in the drift tube and also to find out if the drift tube has an effect on the mass spectra obtained in different modes. The same test compounds were measured with both the MS only and the continuous ion flow mode keeping the measurement conditions as identical as possible, for example, the voltage differences between ESI needle and curtain plate of MS, and ESI needle and the inlet cup of IMS were identical. The position of the ESI needle was adjusted to provide the highest signal intensity. Comparison of the mass spectra of the test compounds, 2,4-DMP, 2,6-DtBP and TBAI, showed no significant difference in the mass spectra obtained with both measurement modes. In addition, comparison of total ion chromatograms (TIC) measured with and without IMS showed very similar TIC levels for both setups. Thus, the signal losses of ions in the drift tube are minimal and the losses are mainly related to the pulsing nature of the IMS measurements.

Limits of detection (LOD, S/N = 3) for the ESI-IMS-MS device was measured for three test compounds: 2,4-DMP, 2,6-DtBP, and TBAI using three different modes: MS only mode, continuous ion slow mode and selected mobility monitoring mode (SMM) (Table 2 in paper I). The measured LOD’s, in nM range, were not strongly dependent on the instrument configuration and measurement modes. An explanation for this is that even though the analyte signal is decreased in the dual gate operation mode compared to that of continuous flow mode, the background is also reduced in the dual gate operation mode by about the same amount.

An important parameter, which characterizes separation efficiency, the resolving power of the developed ESI-IMS-MS device, was measured at room temperature and atmospheric pressure conditions using the same test compounds as discussed in the paragraph above. The resolving power obtained for the ESI-IMS-MS instrument was 80 for 2,6-DtBP under the drift field of 333 V/cm and 200 μs ion gate opening time. Whereas, with the ESI-IMS-FP instrument a resolving power of 95 under the drift field of 378 V/cm and 100 μs ion gate opening time for 2,6-DtBP was obtained (III). The ESI-IMS-MS measurements also showed that with the shorter ion gate opening times better resolution can be obtained as expected. In addition, it was
observed that the drift gas flow has an effect on resolution, for example a resolving power of 31 was obtained with a 0.65 L/min flow rate compared to the value of 47 obtained at 1.3 L/min flow rate for the M⁺ ion of tetrabultammonium iodide (I).

Table 3. Resolving powers measured for TAAH compounds at different gate opening times using ESI-IMS-FP. Experimental values are averages of six repetitions measured on the same day. Relative standard deviations are presented in the brackets. Resolving powers calculated using Equation 9 are also shown. (from Paper III)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gate opening time (µs)</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental/calculated resolving power</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetraethylammonium</td>
<td>93(11%)/114</td>
<td>81(10%)/83</td>
<td>69(10%)/62</td>
<td>41(3%)/40</td>
<td></td>
</tr>
<tr>
<td>Tetrapropylammonium</td>
<td>111(10%)/119</td>
<td>88(2%)/92</td>
<td>77(5%)/71</td>
<td>51(4%)/47</td>
<td></td>
</tr>
<tr>
<td>Tetrabutylammonium</td>
<td>105(11%)/123</td>
<td>93(5%)/99</td>
<td>86(3%)/79</td>
<td>56(4%)/54</td>
<td></td>
</tr>
<tr>
<td>Tetrapentylammonium</td>
<td>111(13%)/125</td>
<td>104(16%)/105</td>
<td>91(9%)/86</td>
<td>63(9%)/60</td>
<td></td>
</tr>
</tbody>
</table>

The effect of ion gate opening time on resolving power was studied in more detail using the IMS-FP instrument with ESI and the TAAH compounds as test compounds (III). Examples of the results are presented in Table 3 together with the calculated resolving powers. Calculations were done using Equation 9 [164, 189], where the K values were replaced with the measured reduced mobility $K_0$:

$$R_i = \frac{L_d^2 / K V_d}{\sqrt{t_g^2 + (16kT \ln 2/V_d e z)(L_d^2 / K V_d)^2}}$$  (9)

where $t_g$ is the initial ion pulse width, k is Boltzman’s constant, e is the elementary charge, z is the number of charges on the ion, $V_d$ is the voltage drop across the drift space, K is the mobility of the ion, and $L_d$ is the drift length.

For many of the TAAH compounds, the experimental resolving power was around 100 at best and showed decreased values when the ion gate opening time increased, as expected. When the experimental values were compared to the calculated resolving power values, the experimental resolving power exceeded the calculated resolving power in most cases when both the 300 µs and 500 µs gate opening times are considered (III). This phenomenon can be explained by a larger contribution of the effect of ion swarm initial shape than diffusion broadening at longer gate opening times. In addition, the calculations do not consider that the time-widths of ion packets are a little shorter than the injection times, which was observed in the previous simulations (I).

6.1.3. Ion mobility measurements with IMS-MS and IMS-FP

Performance of the developed ESI-IMS-MS device was demonstrated by measuring mobilities of 2,4-DMP, 2,6-DtBP, and TBAI using the mobility scan dual-gate mode (I). Figure 23 shows results of this experiment, in which 2,4-DMP and 2,6-DtBP were monitored as protonated molecules and TBAI as the M⁺ ion. The
extracted individual mobility spectra for TBAI and 2,6-DtBP are very simple, with only one peak due to the ion measured. Whereas, the mobility spectrum of 2,4-DMP is more complex and it was presumed that the individual peaks are due to protonated molecule, proton-bound dimer and a proton-bound trimer. The reported calculated reduced mobilities of 2,4-DMP 1.93 cm$^2$V$^{-1}$s$^{-1}$ and 2,6-DtBP 1.54 cm$^2$V$^{-1}$s$^{-1}$ were in relatively good agreement with data reported by Eiceman et al. [190]: 1.93 and 1.45 cm$^2$V$^{-1}$s$^{-1}$ for 2,4-DMP and 2,6-DtBP respectively. The measured reduced mobility of TBAI 1.32 cm$^2$V$^{-1}$s$^{-1}$ (I) is in relative good agreement with the values measured later (Table 4) (II, III).

In this study, the stand alone ESI-IMS-FP instrument was tested by measuring mobilities for 2,6-DtBP and tetraalkylammonium halides using positive ESI and for ortho-phthalic acid (III) using negative ESI. The positive ESI results are discussed in Chapters 6.1.2 and 6.1.4. Figure 24 shows the mobility distribution measured for ortho-phthalic acid using negative ion ESI-IMS-FP. Two clear peaks were observed, one identified as the monomer peak with mobility of 1.69 cm$^2$V$^{-1}$s$^{-1}$ and a smaller peak identified to be a dimer with a mobility of 1.19 cm$^2$V$^{-1}$s$^{-1}$. In the literature, the [M-H$_2$O]$^-$ ion of ortho-phthalic acid has a reported mobility of 1.77 cm$^2$V$^{-1}$s$^{-1}$ is reported [191], which corresponds relatively well with the mobility of the larger peak obtained in this study.

Subsequently both the IMS-MS and IMS-FP instrument has been used successfully in IMS studies [24, 192-194].

Figure 23. Ion mobility spectra of a mixture of 2,4-DMP, 2,6-DtBP, and TBAI obtained with the ESI-IMS-MS instrument. (a) Multiple mass ion mobility spectrum of the mixture, (b) Mass-selective ion mobility spectrum of 2,4-DMP obtained for the mixture (MH$^+$, m/z 108 monitored), (c) Mass-selective ion mobility spectrum of 2,6-DtBP obtained for the mixture (MH$^+$, m/z 192 monitored), (d) Mass-selective ion mobility spectrum of TBAI obtained for the mixture (M$^+$, m/z 242 monitored) (from Paper I).
6.1.4. Chemical standards for positive ESI with ion mobility spectrometry – mass spectrometry

One of the important outcomes of the development of the IMS-MS instrumentation was its utilization in a study in which a chemical standard for IMS is proposed [II]. Chemical standards with known mobility can be used in ion mobility spectrometry to correct differences observed in the calculated reduced mobility values measured with different instruments and conditions [190, 193]. A relative recent review reports types of compounds used as chemical standards in IMS and important properties of good chemical standards are also discussed [195]. For example, the mobility of a good chemical standard should not be affected by changing experimental conditions (e.g. temperature and electric field) and it should be easily available, pure and stable for long periods of time.

In our group, tetraalkylammonium halides (TAAHs) have been suggested as chemical standards for positive ESI-IMS-MS [II] and also used for characterization of developed instrumentation (III). The TAAH compounds are potentially good chemical standards since they pose low clustering tendency (at µM concentration levels) and only a single mobility peak due to the [M]⁺ ion is observed under the ESI-IMS conditions used. Ude and De la Mora also proposed the use of TAAH compounds as mobility standards for aerosol measurements about the same time [196]. In contrast to our study, they used concentrations at mmol/L levels and observed cluster formation in their ESI-DMA (differential mobility analyzer) analysis.

Figure 25 shows a mobility spectrum measured for a mixture of aliphatic C₂-C₈, C₁₀ and C₁₂ tetraalkylammonium salts using the ESI-IMS-MS (II) and ESI-IMS-FP (III) devices. Note that one of the mobility spectra is presented as the function of drift time and the other one as a function of reduced mobility. With both instruments, all of the TAAH compounds produce a single mobility peak, all the peaks are clearly separated, and good resolution is obtained for the all the analytes. The reduced mobilities of the halides measured with the ESI-IMS-MS and the ESI-IMS-FP devices...
were in good agreement (II,III) as can be seen from Table 4. The potential of the TAAH compounds as chemical standards was shown in the ESI-IMS-MS study, since it was observed that the reduced mobilities of the tested halides were independent of the drift field (range 229 – 384 V/cm) and the temperature (range from room temperature to ~90 °C) under the conditions tested (II). In addition, it was observed that the ESI solvent, weather it was acetonitrile, methanol or water/methanol (1:1) did not have an effect on the reduced mobility values at room temperature (II). Lately it has also been reported that mobilities of the TAAH compounds are not affected by drift gas contaminants [197].

Table 4. Reduced mobilities ($K_0$) of TAAH compounds obtained by ESI-IMS-MS (II) and ESI-IMS-FP (III)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reduced mobilities $K_0$, cm$^2$V$^{-1}$s$^{-1}$ obtained with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESI-IMS-MS (II)</td>
</tr>
<tr>
<td>Tetraethylammonium</td>
<td>1.88</td>
</tr>
<tr>
<td>Tetrapropylammonium</td>
<td>1.56</td>
</tr>
<tr>
<td>Tetrabutylammonium</td>
<td>1.33</td>
</tr>
<tr>
<td>Tetrabutylammonium</td>
<td>1.15</td>
</tr>
<tr>
<td>Tetrahexylammonium</td>
<td>1.02</td>
</tr>
<tr>
<td>Tetraheptylammonium</td>
<td>0.92</td>
</tr>
<tr>
<td>Tetraoctylammonium</td>
<td>0.84</td>
</tr>
<tr>
<td>Tetradecylammonium</td>
<td>0.73</td>
</tr>
<tr>
<td>Tetradodecylammonium</td>
<td>0.67</td>
</tr>
</tbody>
</table>
Figure 25. (a) ESI(+)−IMS/MS mass-selected ion mobility spectrum of an equimolar solution (2 μM in 0.1% HCOOH/ acetonitrile) of aliphatic C₂−C₈, C₁₀ and C₁₂ tetraalkylammonium halides (U_drift=357 V/cm and T=298±3K). Tetraalkylammonium cations ([M]+) at m/z 130.0, 186.1, 242.1, 298.4, 354.5, 410.4, 466.5, 578.7 and 690.8 were monitored (from Paper II). (b) ESI(+)−IMS-FP spectrum of an equimolar solution (2μM in MeOH) of aliphatic C₂−C₈, C₁₀ and C₁₂ tetraalkylammonium halides (1.5 l/min drift gas flow rate, 378 V/cm drift field, and 300 μs gate opening time) (from Paper III).
6.2. Development of atmospheric pressure ionization sources

The following two chapters describe characteristics of different ionization techniques developed for the IMS-FP and IMS-MS instruments. In addition, to the self made ion sources, a commercial electrospray ion source was used in the study.

6.2.1. Development of multi-ion source platform (CD-APCI, APPI, R-APCI)

The main aim of the development of the multi-ion source platform was to build a versatile set-up which allows the use of different atmospheric pressure ionization modes (III), both with the IMS-FP instrument and the IMS-MS instrument. Up to date capability for corona discharge atmospheric pressure ionization (CD-APCI), radioactive atmospheric pressure ionization (R-APCI), and atmospheric pressure photo ionization (APPI) has been built for the multi-ion source platform (III). Its operation was demonstrated by measuring mobility distributions for 2,6-DiBP in the three modes, CD-APCI, R-APCI and APPI and comparing these results to the ones obtained with a commercial IonSpray ion source mounted into the IMS-FP instrument. Figure 26 shows mobility distributions measured for 2,6-DiBP using the IMS-FP with different ionization techniques. As it can be seen from the mobility distribution with all the ionization modes only one major mobility peak with relatively good signal-to-noise ratio is observed. The reduced mobility of 2,6-DiBP for different ionization modes was in very good agreement, \( K_0 \) being ~ 1.48 cm\(^2\)/V, with a relative standard deviation for single day measurements below 1% (III). Mobility resolution obtained with all the methods was also very good, around 90-100 depending on the ionization methods, and agreed reasonably with the calculated resolving power (~116).

6.2.2. Desorption/ionization on silicon (DIOS)

The ion source set-up for DIOS was tested with both the IMS-MS and IMS-FP instruments (IV). Three test compounds tetrapropylammonium iodide, tetrabutylammonium iodide and tetrapentylammonium iodide were used with both instruments. Measurements were done either for individual compounds or for mixtures of a tetraalkylammonium halide and 2,6-DiBP. Some measurements were also done for 2,6-DiBP alone. In the following discussion, some data not presented in the publication IV is also discussed.

DIOS measurements were tested with the IMS-MS instrument utilizing two measurement modes, mobility scan dual gate mode (I) and mobility scan laser sampling mode (IV). In the mobility scan dual gate mode only \([M]^+\) ions of the TAAH compounds were monitored. The dual-gate mode produced unstable and badly reproducible ion signal at very low levels with poor peak shapes. However, mobilities of all test compounds could be obtained from a single set of measurements for each analyte. In order to improve the shape of mobility distribution and to increase signal level, four mass-selected mobility spectra of each test compound were summed. The summed mobility spectrum was also smoothed by a moving average function. This procedure produced mobility peaks for the individual halide compounds with different mobilities (Table 5). The data collection was a highly time consuming task, especially since separate sample needed to be used for every mobility spectrum. A typical recording time for the summed mobility spectrum of an individual compound was about 60 minutes.
In order to improve signal intensity by increasing ion sampling efficiency, IMS-MS mobility scan laser-sampling mode was used. The actual drift length (maximum is 21 cm, sum of drift region and desolvation region) varies slightly between experiments due to the fact that the pSi sample area and laser spot are not always at the same position. In order to increase the accuracy of mobility determinations, the mobility of 2,6-DiBP was measured at the same time as the mobility of an individual TAAH compound, and the actual reduced mobility of a TAAH compound was calculated (Table 5) based on the 2,6-DiBP drift time and 2,6-DiBP reduced mobility value taken from previously measured data (III). A rounded value of 1.50 cm²V⁻¹s⁻¹ for [M+H]⁺ ion was used. Figure 27 shows examples of mass-selected mobility spectra of a halide and 2,6-DiBP mixtures.

The mobilities recorded with both of the measurement modes used with the DIOS-IMS-MS set-up agree well with those previously reported for ESI-IMS-MS (II). With the mobility scan dual-gate mode, agreement was better, but obtaining a decent signal required summing of individual measurements and took a long time, therefore the mobility scan laser-sampling mode is preferred.

Ion mobility spectra for the three TAAH test compounds were also measured using the IMS-FP instrument with the DIOS ionization method (Figure 28). The example ion mobility spectra shown have two clearly separated mobility peaks in contrast to the IMS-MS results presented above. Based on the IMS-MS
measurements, the first peak in all three mobility spectra was identified to be due to the \([M]^+\) ion of the corresponding halide. The second peak (the peak with higher mobility than the first peak) is suggested to be due to a fragment ion based on the IMS-MS measurements made using the mass of the fragment ions (formed by a loss of one of the alkyl chains) of the individual TAAH compounds. Resolution of the halide peaks (fragment and \([M]^+\)) obtained with the IMS-FP was at best around 50-70, better than obtained with DIOS-IMS-MS measurements (IV). Due to ion swarm widening in inhomogeneous electric field at Si surface, achieved resolution is below theoretical resolution limit (IV).

Mobilities of the TAAH compounds measured with IMS-FP were estimated using the 2,6-DtBP as an external standard (Table 5). The obtained mobility values agree relatively well with the values obtained by the IMS-MS measurements and the literature values (IV).

Table 5. Reduced mobilities for the tetraalkylammonium halide peaks obtained by DIOS-IMS-MS (IV), DIOS-IMS-FP (IV) and values for ESI-IMS-MS (II).

<table>
<thead>
<tr>
<th>Molecule</th>
<th>DIOS-IMS-MS, ([M]^+, K_0, \text{cm}^2/\text{V}s) (mobility scan dual gate mode)</th>
<th>DIOS-IMS-MS, ([M]^+, K_0, \text{cm}^2\text{V}^{-1}\text{s}^{-1}) (mobility scan laser gate mode)</th>
<th>DIOS-IMS-FP, ([M]^+, K_0, \text{cm}^2\text{V}^{-1}\text{s}^{-1}) (mobility scan dual gate mode)</th>
<th>ESI-IMS-MS, ([M]^+, K_0, \text{cm}^2\text{V}^{-1}\text{s}^{-1}) (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrapropylammonium iodide</td>
<td>1.55</td>
<td>1.49</td>
<td>1.69</td>
<td>1.50</td>
</tr>
<tr>
<td>Tetrabutylammonium iodide</td>
<td>1.33</td>
<td>1.28</td>
<td>1.47</td>
<td>1.29</td>
</tr>
<tr>
<td>Tetrapentylammonium iodide</td>
<td>1.16</td>
<td>1.10</td>
<td>1.31</td>
<td>1.12</td>
</tr>
</tbody>
</table>

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Figure 27. Mass-selected ion mobility spectra of a) tetrapropylammonium halide and 2,6-DtBP, b) tetrabutylammonium halide and 2,6-DtBP (from Paper IV) and c) tetrapentylammonium halide and 2,6-DtBP (1.5 l/min drift gas flow rate, 316 V/cm drift field, and 500μs exit gate opening time).

Figure 28. Ion mobility distributions of a) tetrapropylammonium halide, b) tetrabutylammonium halide, and c) tetrapentylammonium halide (from Paper IV) obtained with IMS-FP. (1.5 l/min drift gas flow rate, 316 V/cm drift field). Mobility scales are adjusted by [M]+ ion peak mobility.
6.3. Coupling of aspiration type ion mobility spectrometer with mass spectrometer

A prototype of the commercial aspiration type ion mobility spectrometer IMCell™ was for the first time coupled to a commercial triple quadrupole mass spectrometer in order to obtain fundamental information of the IMCell™ device (V). In the Experimental section (see Chapter 5.2.6) and in the original manuscript (V) details of the instrumental set-up are presented. The instrumental parameters were initially optimized using 2,6-DtBP as test compound, because it is a chemical standard used in IMS and easily forms a protonated molecule [190]. Operation of the system was shown using 2,6-DtBP and DMMP as model compounds (V).

The first step was optimization of the incoming (sample flow) and outgoing (waste flow) gas flow rates of the IMCell™ device. It was expected that there should be an optimum value of sample/waste flow ratio for the constructed AIMS-MS instrument for both peak shape and signal intensity. Several sample/waste flow ratios were tested and it was observed that the optimum sample/waste flow ratio was about 1.08. Higher sample/waste flow ratios produced both increased peak width and increased signal intensity, signal instability was observed when the ratio was larger than 1.2. Whereas, at a sample/waste flow ratio smaller than 0.9, decreased signal intensity was observed. An explanation for these observations is that higher sample gas flow helps to push ions through the hole in the collecting electrode in the direction of the mass spectrometer, but lower sample gas flow leads the curtain gas, that is flowing from the interface to the IMCell™, to prevent passage of the ions through the hole, especially since there is only a weak electric field within the IMCell™. In addition, it was observed that the increase of sample flow but keeping at the same time the sample/waste flow ratio constant, higher sensitivity could be obtained.

The next step optimized the electric field strength between the IMCell™ and the orifice plate of the mass spectrometer. The field strength was varied by changing of the Ubase voltage in the range from 20 to 100 volts in steps of 10 volts, while the orifice plate of mass spectrometer was maintained at 30 volts. Whereas, the Ubias was varied between 0 and 5 volts in steps of 0.1 volt at every Ubase voltage. Based on the optimization a Ubase value of 30 volts was used in the following measurements, since the 2,6-DtBP signal intensity (m/z 192) was at the highest level around with all the Ubias values when Ubase value was around 30 volts.

Finally, mass-selected ion mobility signals, as a function of bias voltage, were measured for 2,6-DtBP (Figure 29) and DMMP (Figure 30). Protonated molecule of 2,6-DtBP at m/z 192 and a monomer and proton-bound dimer of DMMP at m/z 125 and 249 were measured while increasing Ubias from zero to 5 volts in 0.1 volt steps. The signal maximum for both of the compounds was almost the same, at Ubias voltage of 1.7±0.2 volts for 2,6-DtBP and at of 1.8±0.2 volts for DMMP, however the shapes of the peaks obtained were different.
Figure 29. Six replicated measurements (each presented using a different symbol) of mass-selected ion mobility signals of 2,6-DtBP (SIM of MH$^+$ at m/z 192) obtained with $U_{\text{base}}$ of 30V and the sample/waste flow rate of 1.08 (from Paper V).

Figure 30. Three replicated measurements (each presented using a different symbol) of mass-selected ion mobility signals of DMMP (MIM of MH$^+$ at m/z 125 and M$_2$H$^+$ at m/z 249) obtained with $U_{\text{base}}$ of 30V and the sample/waste flow rate of 1.08. (from Paper V)
7. Conclusions

The developed drift tube IMS is easy to mount to a commercial mass spectrometer. The design of the device allows also easy usage of various API sources, which can be standard commercial ion sources (some modifications needed) or custom-made ion sources. Due to the minimal loses of ions during ion transfer through the drift tube, the detection limits obtainable with the IMS-MS instrument are equivalent to detection limits of the commercial triple quadrupole mass spectrometer alone. Thus, it is expected that very low detection limits could be obtained if a new generation triple quadrupole mass spectrometer was used in the combination. A clear limitation of the instrument is that real-time mobility spectra in single gate operation could not be realized with the quadrupole mass spectrometer (1). The current IMS-MS instrument provides high mobility resolving power, a resolving power of 80 was demonstrated. As a result, the IMS-MS can be utilized for analysis of complex samples in many applications, such as isomer separation studies, drug discovery and environmental screening. Development of the IMS-MS instrument also led to the proposed use of tetraalkyammonium ions as chemical standards for ESI-IMS. The instrument also potentially provides possibilities for structural studies after mobility separation, since the mass spectrometer used is a triple quadrupole mass spectrometer.

The developed standalone IMS-FP instrument with different ionization modes demonstrated somewhat better resolution than was obtained with the IMS-MS device. Resolving powers of around 100 at ambient temperature and atmospheric pressure were obtained, and excellent reproducibility of mobility measurements was achieved with all ionization techniques used (ESI, R-APCI, CD-APCI, and APPI). The multi-ion source platform developed for the IMS-FP instrument allows fast switching between various ionization methods. It can also be used in the IMS-MS instrument to enhance the capabilities of this instrument. Thus, with both the IMS instruments, a wide range of compounds (ionic and neutral, polar and non-polar, in liquid and gas phase) can be analyzed either in positive ion mode or in negative ion mode.

A clear advantage of the IMS-FP instrument compared to the IMS-MS instrument is in the total measurement time. With the IMS-FP instrument, a good quality mobility spectrum can be obtained in couple of minutes, however the IMS-MS instrument takes in the order of 10-20 minutes. Although, analysis time of the IMS-MS instrument can be shortened to an IMS-FP time scale by replacing the triple quadrupole mass spectrometer with a time-of-flight mass spectrometer.

A simple DIOS ion source set-up was built for the IMS-MS and IMS-FP instruments. This set-up was tested with three of TAAH standard compounds in three different operation modes, two with the IMS-MS instrument (mobility scan dual gate mode and mobility scan laser gate mode) and one with the IMS-FP instrument (both ion gates were open, the drift time was measured from laser shot). The reduced mobilities measured with all tested modes were in good agreement with published data previously obtained for the TAAH standards with ESI-IMS-MS. However, a number of improvements are needed in order to reach the full potential of the DIOS method, such as utilization of laser with higher repetition rate than was used and building of advanced ion optics of the source that would most likely allow
better ion extraction and transfer into drift tube and therefore clearly better sensitivity. Laser ionization based IMS analysis is believed to allow fast screening analysis in many application areas.

The five ionization methods reported in this study, which can be used with the developed IMS-FP and IMS-MS instruments, allow analysis of very wide range of compounds. It is also expected that other ionization methods, if needed, are easy to implement to the instruments due to the multi-ion source platform. This flexibility and ability to use different ionization techniques on the same drift tube is not typical for the commercial IMS instruments. Thus, the developed IMS instruments have a wide area of potential applications. The developed drift tube is robust and relative easy to use when taken into account that the instruments are custom-made. The first version of the electronics was sensitive to electrical discharges, which could appear in the ion source and ion gates. However, this problem was solved by increasing of robustness of the electronics. Mobility resolution obtained with both the instruments is better than the typically reported values for commercial IMS instruments. In addition, the experimental resolving powers agree relatively well with the theoretically calculated ones.

A prototype of the commercial aspiration ion mobility spectrometer, IMCell™, was combined, for the first time, with a mass spectrometer. Interfacing of the IMCell™ to API-mass spectrometer was a relatively simple task. With the built interface plate, mounting of IMCell™ to the API-mass spectrometer is fast and no major modification to the mass spectrometer is required. Performance of the combination was demonstrated by measuring mobility distribution for 2,6-DnBP and DMMP. These measurements showed that the IMCell™-MS combination can be used for gathering fundamental information of the AIMS device. One possible application of the combination could be the use of AIMS as a pre-filter to improve signal-to-noise rations as already demonstrated for the DMS device while mounted in front of a mass spectrometer.
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Appendix: Papers I - V