4. EXPERIMENTAL PLAN

As mentioned above, our initial experiments must focus on the design, stabilization, and quantitative evaluation of an instrumental platform for tandem DMS measurements. Then studies in chemical orthogonality by ion modification in air at ambient pressure, through ion-cluster formation and ion fragmentation by electric field heating, can be made with a thoroughly documented test platform. Issues of technology to build a reliable tandem DMS are given in section 4.1 and experiments involving ion chemistry and chemical orthogonality are detailed in sections 4.2 to 4.4,.

4.1. Tandem DMS instrument

4.1.1. Ion purification for stage one DMS

In DMS instruments with atmospheric pressure chemical ionization (APCI) sources, sample vapors are passed through an ionizer (a ^{63}Ni foil) directly into the DMS analyzer. Although resulting measurements are often visibly free of matrix effects, neutral unreacted sample flow along with reactant and product ions through the analyzer. Our studies with DMS/DMS/MS demonstrated that these unreacted vapors form cluster ions in the supersonic expansion region of the MS interface significantly complicating data interpretation. There was a concern that these vapors could compromise ion motion in the second DMS stage. To avoid complications of mixed composition clusters in stage two or when combined with a mass spectrometer, we wish to pass ions through stage one of the tandem DMS in a purified gas atmosphere.

We developed a curtain gas filter (CGF) based on mobility separation of ions from neutrals and use as a filter between an ESI source and mass spectrometer to protect the mass spectrometer from phosphate buffers and silica.[40] A CGF inlet will be integrated into a single DMS instrument and demonstrated; later we will combine with our DMS/DMS instrument. A ^{63}Ni foil has functioned with the CGF on a mass spectrometer with enough ion flux to anticipate acceptable signal strength in a DMS or DMS/DMS instrument.

4.1.2. Design of DMS/DMS (Faraday plate unit and DMS/DMS/MS unit)

Our current tandem DMS instrument which is a second generation proof-of-concept design built in 2012, lacks most of the essential control of utilities needed in a research grade instrument. We intend to refine the design of DMS/DMS with a new drift tube, improved control of parameters, and thoroughly document precision and accuracy of parameters. Drift tubes for DMS/DMS are relatively small (Fig. 9) and inexpensive to build, at least two drift tubes will be built, one equipped with Faraday plate detector and one to attach to a mass spectrometer. Hardware for utilities including mass flow controllers, fitted ovens, and electronic controls for separation voltage, compensation voltage, and small current amplifiers, restrict us to two test beds. These will include computer control of parameters and data acquisition and easy in-easy out arrangement for other tandem DMS drift tubes which may arise in later stages of the project and also the triple stage DMS (section 4.4). Specifics on dimensions of the DMS/DMS are shown in Figs. 1 & 9. The analyzer is comprised of a pair of ceramic plates with gold coated copper plate, 13 mm long and 5 mm wide. The plates are separated by 0.5 mm

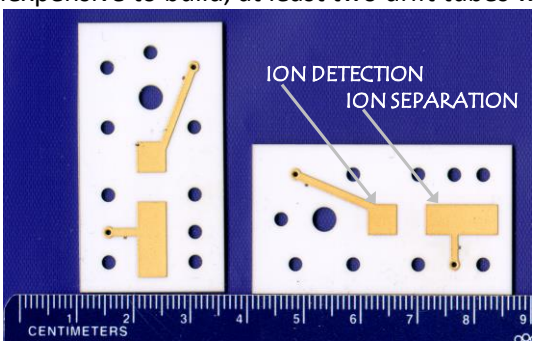


Figure 10. Ceramic plates with metalized areas for use in a DMS drift tube. Two plates are placed together and separated with a 0.5 mm Teflon gasket that also defines ion flow in the center of the assembly.

Teflon spacer and mechanically stabilized in an aluminum frame (Fig. 9). A gap between the plates of DMS1 and DMS2 is 5 mm and the frame includes tube fittings to allow the addition of reagent gas and flow from the ion source.

4.1.3. Instrument temperature

To permit reliable studies of ion-molecule chemistry with thermalized ions, we must know and control gas temperature and this involves placing the tandem analyzer inside a thermostated gas bath and preheating gases before entering the instrument. In our proof-of-concept design, the drift tube was operated at ambient pressure, limiting precision of thermochemical measurements and flexibility to study temperature dependent ion behavior. Temperatures in our first DMS/DMS will be uniform in all regions of the instrument inside a gas bath where we have achieved temperature precision of better than ±2°C throughout the length of 30 cm long conventional drift tubes for IMS. Our goal in this project with such a small analyzer is ±0.5°C.

We expect that the oven will be based on designs and materials found in convention oven yet will be custom built at NMSU for a close form fit with the DMS/DMS drift tube so wires connecting to RF driver electronics can be short with low capacitance. This means a stainless steel inner skin, a resistive coil heater element, and GlassPack insulation and outer shell. The overall dimensions of the oven are planned as 25 cm long x 15 mm wide x 10 cm high. Range of temperature is planned as 0 to 150°C. The cryogenic control is through liquid N_2 direct spray into the oven volume or indirect cooling with gas exchange. Our laboratory operates several GC or GC/MS instrument with commercial of self-built cyro-controlled ovens and we have operated mobility spectrometers at -30C.

4.1.4. Control of gases and handling of flows

Mass flow controllers with computer interfaces are supplied only purified gas to provide the drift tube with sample and reagent gases. Addition of reagents or sample occurs down-flow from the mass flow controllers. Gases are purified in membrane or catalytic generators and polished through 13X molecular sieve to a moisture level of 1 $ppm_{v,}$ as measured by a Panametrics Moisture Image Series 2. Gas flow of 1.5 L/min enters the ion source. Reagent gases are introduced with purified air at 0.2 L/min between the two DMS plates (Figs. 2 & 9), constituting roughly a 12% dilution of the flow from DMS1. The residence times for ions in the first and second DMS instruments were 1ms and 0.9 ms, respectively and the total time ions spend in analyzer is 2.8 ms. The mass flow controllers favored in our team are Fathom Technologies which are interfaced to a computer for independent control by software. All aspects of flow and temperature will come through software regulation with National Instrument interface cards and either Labview or Visual Basic.

4.1.5. Preparation of test substances and concentrations of reagent gases

All substances as received from a commercial source contain impurities and these can sometimes dominate response in APCI based instruments. The only practical method to obtain defensible results in ion molecule studies with an APCI instrument is through pre-fractionation with gas chromatography. Data obtained during a heart-cut of the chromatographic peak can be regarded as authentic and free of impurities, as much as possible. We have already placed a GC inlet on the prototype DMSxDMS as with other DMS or IMS experiments in our laboratory and concentrations of substances delivered to the tandem DMS can range from 10 pg to 100 ng. This occurs over elution of the peak lasting 15 to 20 seconds, too fast to generate a DMSxDMS map (Fig. 4). Consequently, each experiment will be preceded by a measurement of where the location of the product ion in CV_{DMS1} axis is determined. Once known, the separation voltage will be fixed to allow this selected ion to pass DMS1 continuously. The scan rate for DMS2 is 1 s/scan and thus, changes in ΔK will be measured using DMS2 in reference CV_{DMS1} for the ion in purified air.

Vapors of reagents are generated using a syringe pump, at a given liquid delivery rate, with a capillary column feed into a vaporizer. A known rate of liquid flow and dilution gas will generate 1 to 500 ppm levels of reagents in the gas flow introduced between the DMS stages. Flow rates are of sample are 1 to 1.5 L/min and flow rates for reagent gas are 0.2 to 0.3 L/min.

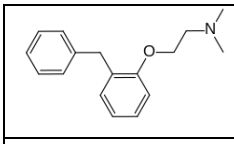
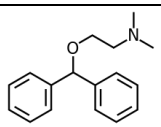
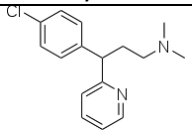
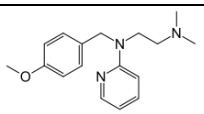
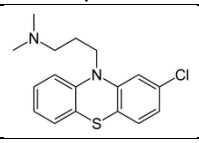
4.1.6. Computer control of utility parameters

In the past two years, we have begun to control all parameters of mobility spectrometers using software and interface cards from National Instruments. Software has been written in Visual Basic. We will need four analog I/O (temp., flows (2x), pressure) to control utilities. Separation voltages, compensation voltages, and detector signals from the DMS DMS will be managed using custom build software and hardward with another computer. Syringe pumps and GC will be controlled manually.

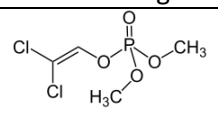
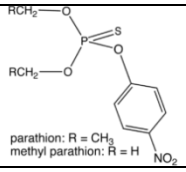
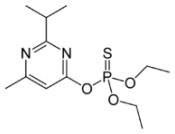
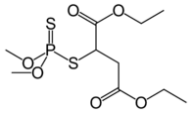
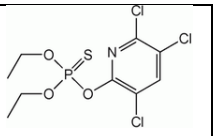
4.2 Adduct formation in DMS x DMS and assessment of chemical orthogonality

In these experiments, five substances spanning a range of molar masses in each of two chemical families will be used to study chemical orthogonality. The substances are:

a. pharmaceuticals where there is common moiety and differing structures and one isobar pair.

				
255.36	255.36	274.79	285.38	318.86
Phenyltoloxamine	Diphenhydramine	Chlorpheniramine	Mepyramine	Chlorpromazine

b. pesticides-a range of substances derived from $R_3P{=}S$ and one $R_3P{=}O$

				
220.98	291.26	304.35	330.36	350.59
Dichlorvos	Parathion	Diazinon	Malathion	Chlorpyrifos

Each has a vapor pressure suitable for pre-fractionation in a GC inlet and ionization chemistry suitable for our APCI ion source. Alternative substances, if needed, include ketones (Fig. 8), amino acids and peptides with an ESI source, used extensively in our group.

4.2.1. Selection of reagent gases

We will employ reagent gases which should form a range of stabilities and structures of ion-adducts based on molecular descriptors of basicity, polarizability, and dipole strength. Pharmaceuticals which have protonated secondary or tertiary amines should form strong adducts with amines and adducts of lesser strength with oxygen bases (alcohols, ketones). Weak or practically no adducts will be formed with chlorocarbons or alkenes although alpha functions may be controlled with high levels of these. The ion-molecule adducts, once mass identified, will be modeled using *ab initio* calculations and compared to experimental findings.

Our plan is to choose, within in each chemical family of modifiers or reagents, a homologous set with some structural variations.

1) Alcohols will include methanol to 1-pentanol, isopropanol, 2-butanol, and 2- and 3-pentanol for each test substance.
2) Ketones will include acetone, 2-butanone to 2 pentanone and methylisopropylketone and methyl isobutyl ketone.
3) Amines will include methyl amine, ethyl amine, propyl amine, dimethylamine, and trimethylamine.
4) Chlorocarbons: methylene chloride, chloroform, 1,2 dichloroethylene

As seen with the preliminary studies for methyl salicylate and isopropanol, the expected product ion of MO_2^-(IPA) was not observed and instead a displacement reaction formed O_2^-(IPA). In the instance of proton chemistry found with nitrogenous pharmaceuticals and phosphate ester pesticides, displacement reactions of this kind should not be observed; however, displacements of M_2H^+ ions to heterogeneous proton bound dimers, i.e., MH^+(IPA), should occur. Stable adduct ions with alkenes and chlorocarbons are not expected although changes in alpha functions may be observed and will be documented. Each combination of sample and reagent gas will be measured, characterized, and evaluated for chemical orthogonality (4.2.4).

4.2.2. Vapor concentration of reagent gases entering the tandem DMS

Vapor concentrations of reagent gases become significant above 50 ppm[33,38] and increase asymptotically, often exponentially until vapor levels result in surface condensation. Tandem DMS experiments will be completed spanning the range of 50 to 500 ppm while all other parameters are constant. Reagent-ion combinations from 25 to 150°C which show chemical orthogonality (a shift in compensation voltage displaced from the original location in the contour plot of ion intensity, CV_{DMS1}, and CV_{DMS2} (Fig. 4) will be carried into subsequent studies. Each combination will be mass identified using the tandem DMS/MS combination.

4.2.3. Effects of temperature and moisture of supporting gas atmosphere

Temperature and moisture in IMS and DMS at ambient pressure are roughly equivalent, in importance, to vacuum in mass spectrometry and will controlled since they govern both the formation of ions from sample and the mobility behavior of ions in DMS. Water can be considered a reagent gas and affects alpha plots in nearly identical patterns as other polar neutrals such as isopropanol. Water has a secondary and unwelcome effect of suppressing response in APCI reactions and moisture levels from 1 to 20 ppm are used routinely in our laboratory. An attractive feature of DMS/DMS is that we can use moisture levels favoring ion formation in the source region (1 ppm) and then add moisture in the DMS/DMS interface at levels from 50 to 500 ppm to probe effects of moisture on ion stability and behavior. These will be backed by calculations on ion structures based on thermochemical values of Kebarle's team on hydration of the gas proton[41] and NIST listings of these or similar substances.

The role or effect of temperature on gas phase ions and ion adducts is complex governing ion energies (a combination of thermal plus field heating). We know that heat flow into an ion is mass dependent and the combination of thermal and field energies will span a relatively large range. At temperatures above 50°C, some proton bound dimers of samples and some ion-reagent adducts will undergo dissociations by field heating although we have no information specific enough to know these. Measurements of adduct ion stability will be determined for combinations of ions and reagents studied in section 4.2.3. Increases in temperature above 100°C initiates ion fragmentation (4.3 below) in some but not all substances and the interest here is to quantitatively explore the stability and fragmentation of ion cluster from 25 to 150°C. We have a capability to use sub-ambient temperatures if needed.

4.2.4. Assessment of Chemical Orthogonality

We regard the DMSxDMS data set (Fig. 8) as multi-way data and results will be arranged in matrix, as in standard multivariate data sets, or in a cube, as with three-way data, where results are arranged with a variable (concentration, temperature, moisture). Our first efforts will be based on PARAFAC to decompose this multi-way data and unfold the array to a matrix. We have unfolded such data previously using software and methods provided by Prof. Synovac and now Prof. Pierce at Univ. Wash and Seattle Pacific Univ, respectively. Using PARAFAC and the customized software we hope to describe the data and score the findings in terms of chemical orthogonality. We are not specialists in this subject and will seek assistance from these colleagues (see letters of support) and have included consultancy funds for this purpose.

4.3 Ion Fragmentation

We will fragment gas ions of the pharmaceuticals and pesticides (4.2) using strong electric fields in the middle region between the two DMS sections. Elevated gas temperatures may be needed. The onset voltages for fragmentation of ions already know follow the trend: alcohols < esters < ketones.

4.3.1. Field strengths and options for ion fragmentation

The onset voltage to fragment ions of protonated tertiary amines (pharmaceuticals) and protonated sulfur-organophosphonates (pesticides) will be determined for each of several chemical reagent gases by generating dispersion plots (Fig. 1 & 6). We have several Sionex SVAC model DMS instrument where reagent gas can be introduced with the analyte and will supplement the demand on DMS/DMS instruments with a GC-SVAC combination. Our objective is precise measures of onset of ion-adduct dissociation and these instruments have been used for similar studies.[22,39] To achieve sample pre-fractionation while obtaining dispersion plots, the GC will be degraded in efficiency with flows/temperatures for chromatography peak widths of 45 to 60 s. We will use high speed low resolution dispersion plots for a preliminary measure of onset of dissociation and will follow with a narrower range for separation voltage to obtain a precise measure of onset of dissociation or fragmentation. Dissociation may occur at comparatively low electric field strengths and fragmentation should occur at strong fields, in excess of 150 Td at 120 to 140°C. If we do not observe fragmentation in the SVAC instrument with an upper range of 178 Td, we will install an Owlstone ultraFAIMS drift tube (Fig. 11) between two stages of an NMSU built tandem DMS. The Owlstone unit can be operated at 320 Td, or fields of 75,000 V/cm and this should be sufficient to fragment ions of 400 Da.

Figure 11. Owlstone ultraFAIMS drift tube which can be placed between DMS 1 and DMS 2 to fragment ions.

4.3.2. Gas temperatures and onset to decluster adduct ions and fragment ions

The compliment to ion heating by electric fields is ion heating by gas temperature. The Owlstone unit will be built into a tandem DMS (Fig. 12) for these studies and with electric fields of 280 to 320 Td should permit determinations on the onset to decluster or fragment ions possibly beginning as low as 30°C to 50°C. Declustering of adducts is anticipated to precede ion fragmentation. Since the electric fields in this unit can be significantly reduced by computer control, the gas temperature for onset of fragmentation will be determined using dispersion plots at certain gas temperatures. We will narrow the range of ion fragmentation from the dispersion plots and then carefully increment temperature, in steps of 1°C in the controlled bath gas oven, for precise determinations of temperatures for on-set to

decluster or fragment ions (4.1.3). The combinations of test substances with reagent gas studied here will come from those with favorable interactions, determined in section 4.2.1

4.3.3. Levels of moisture to decluster adduct ions and fragment ions

The stability of ions and ion-adducts in strong electric fields will be measured using the on-set of dissociation or fragmentation with supporting gas atmospheres, introduced into DMS2 with moistures from 10 to 500 ppm. The method of generating high speed dispersion curves with low efficiency GC inlet, followed with a narrower range for a precise measurement will provide data that discloses quantitatively the role of moisture on ion stability. Experimental findings will be compared with results from computational *ab-initio* calculations on ion structures known from mass analysis and corrected for adducts using thermochemical computations.

4.4 Chemical orthogonality in triple stage DMS through fragmentation and adduct formation

A complete tandem instrument should include capabilities for ion fragmentation, ion cluster formation, and then ion fragmentation followed with ion mobility selection and cluster formation with the ion fragment, before final mobility scanning. This would provide unprecedented analytical specify for low resolving power analyzers at ambient pressure and would introduce ion characterization by physics alone or in combination with a maximum five dimensions of selectivity: ion selection, clustering, fragmentation, reclustering and ion separation.

4.4.1. Modification of tandem instrument with third mobility stage

We will expand the DMS DMS capability with a third DMS section and a second gas inlet for cluster formation between DMS2 and DMS3. The Owlstone ultraFAIMS, for ion fragmentation, will be maintained between DMS1 and DMS2 with a gas inlet directly before the ion "fragmenter". In this advance to such a multi-dimensional instrument, the core design will need expansion to three DMS stages and addition of a second vapor inlet, this between DMS2 and DMS3. This necessitate the such development eventually of a next generation of software and a new electronics package; however investment of time and resources will be delayed until the concept is proven and we will use a hybrid approach where the existing electronics are used to synchronize and control DMS1 and DMS3 and DMS2 is operated in an all pass or single ion pass mode. The drift tube will be developed in the same manner as our existing tandem DMS (section 4.1) where ceramic plates (Fig. 10) will be cut to fit inside the DMS frame. The complete analyzer is shown in Figure 12

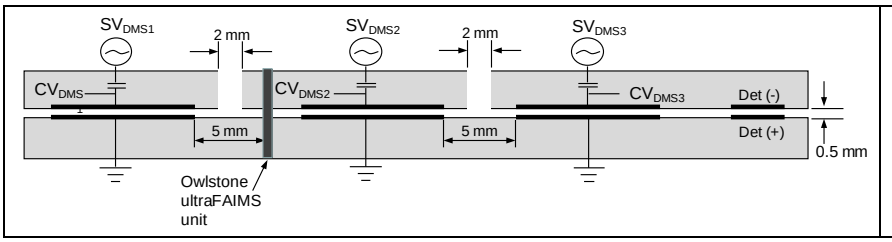


Figure 12. Schematic of triple stage DMS including ion fragmentation and two stages for forming ion clusters with a reagent gas.

4.4.2. Combinations of ion fragmentation and ion clustering of fragments

The model experimental plan we wish to use with the triple DMS instrument is comprised of a protocol to generate data for tandem DMS measurement of a substance.

1). Select ion in DMS1 by control of SV_{DMS1} and CV_{DMS1} and pass the ion to next stage
Option: modify ion by cluster formation with reagent gas.

2). Decluster or fragment ion in an Owlstone ultraFAIMS unit.
3). Isolate or select ion in DMS2 by control of SV_{DMS2} and CV_{DMS2} and pass the ion to next stage
Option: modify ion by cluster formation with reagent gas.
4). Separate and characterize ions in DMS3 using dispersion plots (sweeps of of SV_{DMS3}) or select specific ions to pass to the detectors.

The number of combinations of experimental parameters within the triple DMS instrument is large and experiments with the unit shown in Fig. 12 will be directed by discoveries generated in sections 4.2 and 4.3. Our goal will be to measure overall specificity or chemical orthogonality possible with a triple stage instrument where the total of capabilities listed above can be brought into an ion mobility measurement.