Appendix A. The design and implementation of a single-droplet source for field-induced droplet ionization mass spectrometry

A.1. Introduction

Single droplet mass spectrometry (MS) is achieved through field-induced droplet ionization. Applications of single droplet MS are discussed in Chapters 8 and 9, but these experiments represent only the initial stages of exciting and new applications for chemistry and the subsequent analysis of single droplets. This appendix is written with the intention that others may employ and even improve the designs and explore single droplet chemistry. Design considerations and safety requirements are presented and discussed for the single droplet FIDI source.

A droplet is mechanically suspended between a plate electrode and the sampling capillary of an ion trap mass spectrometer. A high voltage pulse on the electrode applies a strong electric field to the droplet resulting in the ejection of jets of charged progeny droplets. The design of an idealized single droplet FIDI source requires knowledge of the dynamics of the event including field strengths and the timescale over which FIDI occurs. Section A.2 discusses the theory and equations governing design considerations. Section A.3 presents drawings of the instruments, relevant electronic schematics, and mass spectrometer modifications. Sample positive ion mass spectra from a single droplet containing α lactalbumin are shown in Section A.4.

A.2. Theory and factors affecting design

As is mentioned throughout this thesis, field-induced droplet ionization occurs in droplets upon the application of a strong applied electric field. This field need not be constant or linear, and investigations into the FIDI dynamics establishes the critical field strength and duration for a droplet of known size and bulk physical constants. Excess electrical pressure leading to FIDI develops in droplets of radius r and surface tension σ when the applied field exceeds a critical value, E_c known as the Taylor limit eq (A.1), named for G. I. Taylor who pioneered the corresponding theory.[1-3]

$$E_c = \frac{1.625}{(8\pi)^{1/2}}\left(\frac{2\sigma}{\varepsilon_0 r}\right)^{1/2} \tag{A.1}$$

Above the Taylor limit, droplets develop two opposing conical tips that emit fine jets of oppositely charged progeny droplets. Below the Taylor limit, droplets undergo field-dependent shape oscillations that dampen out to an equilibrium prolate elliptical shape as predicted by Taylor. Recent investigations in our laboratory demonstrate that the timescale of droplet elongation, tip formation, and progeny droplet generation slightly above the Taylor limit is related to the timescale of the oscillations slightly below the Taylor limit. The timescale to initiate FIDI, τ_{FIDI} is approximately 75% the time of an oscillation period, ν^{-1}, as the applied field approaches the Taylor limit as noted by eq (A.2).[4]

$$\tau_{\mathrm{FIDI}} \approx 0.75\left(\nu_{E\to E_c}\right)^{-1} \tag{A.2}$$

Thus, models of sub-critical shape oscillations become an important predictor for the timescales of FIDI. In exploring the oscillations of 225 μm methanol droplets below the

Taylor limit, we found good agreement between the experimental shape oscillations and the numerical model of Basaran and co-workers as well as the analytical model of Feng and Beard. Feng and Beard model the oscillation frequency ν_{FB} as a function of the applied field, and the droplet's natural $l = 2$ mode oscillation shown in eq (A.3).[5]

$$\nu_{FB} = \frac{8^{1/2}}{2\pi}\left[1 - 2.764E^2\left(\frac{\varepsilon_0 r}{2\sigma}\right)\right]\left(\frac{\sigma}{r^3\rho}\right)^{1/2} \tag{A.3}$$

The frequency decreases proportionally with E^2 until the Taylor limit is reached, at which point the frequency is given by eq (A.4).

$$\nu_{FB}(E_c) = \frac{8^{1/2}}{2\pi}\left[1 - 2.764\frac{1.625^2}{8\pi}\right]\left(\frac{\sigma}{r^3\rho}\right)^{1/2} \tag{A.4}$$

Equations (A.2) and (A.4) may be combined into eq (A.5) to show an approximate timescale for FIDI as a function of droplet size, density, and surface tension.

$$\tau_{FIDI} \approx 2.3\left(\frac{r^3\rho}{\sigma}\right)^{1/2} \tag{A.5}$$

Equations (A.1) and (A.5) are critical when designing a FIDI source. Likewise, other design factors must be considered regarding the size and separation of the electrodes that establish the electric field. When using a parallel plate capacitor configuration, the electric field is the familiar $E = V / d$ where V is the voltage difference and d is the distance between the electrodes. If design constraints limit the maximum voltage to a value V_{max}, the maximum plate spacing is likewise limited to a value d_{max}, shown in eq (A.6). Here the field necessary for FIDI from (A.1) represents the minimum field that restricts d_{max}.

$$d_{max} = \frac{2V_{max}}{1.625}\left(\frac{\pi\varepsilon_0 r}{2\sigma}\right)^{1/2} \tag{A.6}$$

As expected, higher voltages afford a greater plate separation. Equation (A.6) also suggests the maximum plate separation increases with $r^{1/2}$ because larger droplets require lower field strengths for FIDI. Unfortunately, the dielectric breakdown limit of air and non-linear field effects also limit plate spacing. For instance, the breakdown of air is ideally ~30 kV cm^{-1}, however arcing often occurs at lower field strengths.

Similarly, the droplet itself will distort the electric field. These distortions may be minimized by maximizing the ratio between the plate spacing, d, and the droplet diameter, $2r$, which is derived from eq (A.6).

$$\frac{d_{\max}}{2r} = \frac{V_{\max}}{1.625}\left(\frac{\pi\varepsilon_0}{\sigma r}\right)^{1/2} \qquad \text{(A.7)}$$

Although (A.1) predicts that larger droplets require lower fields, eq (A.7) shows that smaller droplets will minimize the field distortions and maximize the plate spacing to droplet size ratio proportionally with $r^{-1/2}$.

Thus when designing a FIDI source, it is important to consider droplet size, E_c, $V_{\max}$ and τ_{FIDI}. The timescale is important because it determines what electric schematics are necessary for rapidly switching the electric fields. Generally, droplets in the micron size regime have FIDI timescales on the order of tens to hundreds of microseconds. At this speed high voltage solid state switching transistors are necessary to rapidly switch on and off the fields. Larger, millimeter-sized droplets have FIDI timescales on the order of milliseconds. This longer timescale allows the replacement of high voltage transistor circuits with reed relays and more modest electronics.

A.3. Instrument design and description

The single droplet source is mounted in front of the Beauchamp group LCQ Deca (ThermoFinnigan) ion trap mass spectrometer. All discussions are geared towards this implementation and should be taken into consideration when designing a source for a different mass spectrometer.

A.3.1. FIDI hardware

Figure A.1 shows an illustration of the apparatus mounted relative to the LCQ capillary inlet. The FIDI source consists of multiple parts which are all mounted on a 5 by 7 inch acrylic sled that sits on the rails designed for the commercial electrospray source. The FIDI region is defined by a parallel plate electrode and the capillary inlet itself. A mechanically suspended droplet bisects the field. Frame B of Figure A.1 shows this region in greater detail.

The droplet hangs from a 28-gauge stainless steel tube (HTX-28-24, Small Parts, Inc.). The tube connects to a sample line and the syringe through Upchurch Scientific fittings. The solution to be analyzed is fed through the tube until a 1-2 mm diameter droplet forms at the bottom of the tube. The tube and fittings are mounted on a small three-dimensional stage that adjusts to direct the progeny jets into the capillary inlet of the LCQ. The parallel plate electrode is also mounted on an independent three-dimensional stage allowing precise control of d, the spacing between the electrode and

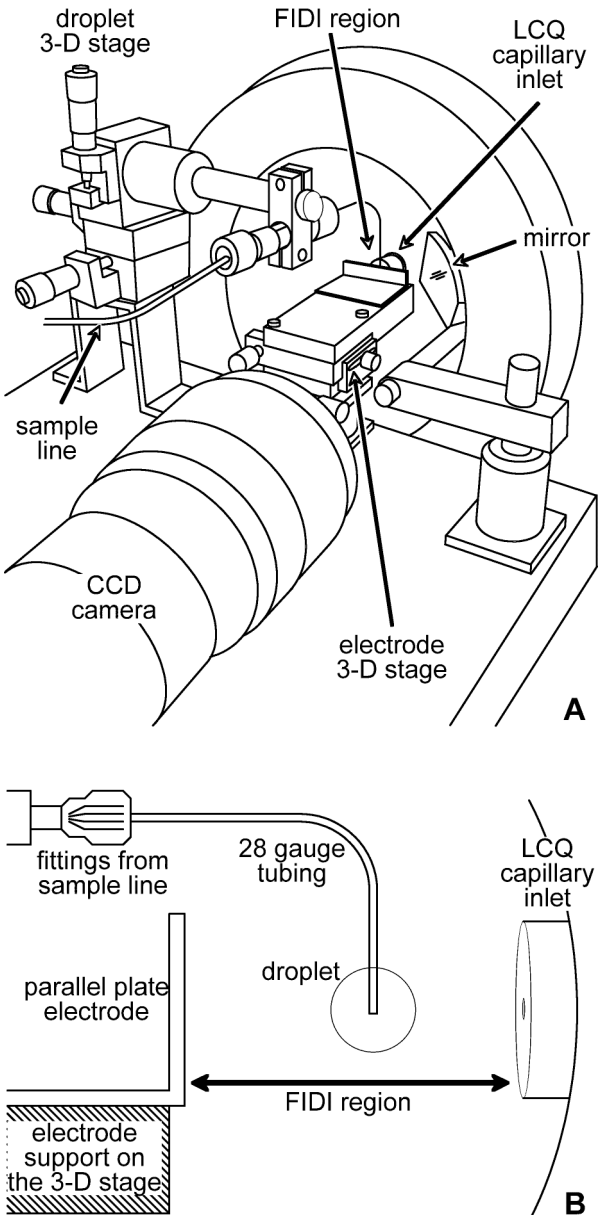

Figure A.1. Diagram of the single droplet FIDI-MS setup. Frame A shows a perspective illustration of the source relative to the capillary inlet on the LCQ mass spectrometer. A sample line delivers solution and analyte to a 28-gauge hypodermic tube forming a droplet on the bottom. At the bottom of the tube, the droplet is mechanically suspended between the LCQ capillary inlet and a parallel plate electrode which establish the FIDI field (Frame B). Both the tubing and the electrode are on separate three-dimensional stages. A mirror and CCD camera enable side-on visualization of the FIDI region.

the capillary. The FIDI region is monitored with a CCD camera and a mirror which are each mounted on the sled. The camera allows a side-on view of the FIDI region that assists with aligning the droplet relative to the electrode and capillary and provides information on droplet size.

Droplets in this study are roughly 1-2 mm in diameter. This is an important parameter for determining the field strengths and durations needed to achieve jetting and progeny formation. At this size, eqs (A.1) and (A.5) predict $E_c \sim 13$ kV cm^{-1} and $\tau_{FIDI} \sim 9$ ms for aqueous droplets. For a typical organic droplet where $\sigma = 0.020$ N m^{-1} and $\rho = 800$ kg m^{-3}, these equations predict $E_c \sim 7$ kV cm^{-1} and $\tau_{FIDI} \sim 15$ ms.

Under initial conditions, the capillary, tubing and electrode are maintained at electrical ground. The liquid sample is fed through the tubing to establish the proper droplet size in a field-free environment. At a user-defined point, an electric field is established using two high voltage (HV) switching circuits. The electrode voltage switches from ground to HV and the tubing voltage switches from ground to HV/2. These voltages remain for a time $t \sim \tau$ before returning to ground. Throughout this time, the capillary remains at electrical ground. Typically HV = 7-9 kV and HV/2 = 3.5-4.5 kV. When sampling positively charged droplets into the mass spectrometer for a positive ion mass spectrum, both HV and HV/2 deliver positive voltage. Conversely, negative high voltage settings direct negatively charged progeny into the instrument for a negative ion mass spectrum. Following the time *t*, the electrode and hypodermic tube return to electrical ground. The parent droplet remains mechanically suspended and may be sampled by FIDI repeatedly until the volume of the droplet decreases such that the applied field is below the Taylor limit given by eq (A.1).

A.3.2. Electronics

The millisecond timescales required for FIDI of 1-2 mm droplets simplifies the electronic circuits. Previous investigations on the dynamics of jetting and progeny formation focused on the behavior of 225 μm diameter methanol droplets. For these droplets, FIDI timescales are between 400 and 700 μs depending on the applied field strength. Such timescales require custom circuitry and high-voltage MOSFET switches to achieve rapid ($\leq$ 1 μs) switching of the high voltage. In the case of 1-2 mm droplets, solid state switching circuitry may be replaced with commercial high voltage reed relays.

Figure A.2 shows the electrical schematic for the experiment. In frame A, a 74LS123 monostable vibrator generates a 5V pulse when a pushbutton switch SW1 is closed by the operator. The capacitor C_{ext} = 30 μF and the R_{ext} = 10 kΩ potentiometer determine a pulse length between 1 and 10 ms. The waveform passes through two inverting Schmitt triggers that are inside a single 74LS14 package. The Schmitt triggers provide a test point (TP1) for a digital oscilloscope that monitors the waveform and pulse length, and proved the necessary current to drive the switching circuitry.

Frames B and C are each a switching circuit connected to the FIDI 28-gauge tube and electrode, respectively. Both switches consist of a 6N135 optoisolator and a 2N6388 Darlington transistor to drive the reed relay from the pulse provided by the monostable multivibrator. Figure A.2B shows the HV/2 switch for the 28-gauge tubing. The input $HV/2_{in}$ refers to an external power supply delivering between 3.5 and 4.5 kV. The circuit uses a Magnecraft W102VX-50 reed relay capable of switching up to 5 kV. The

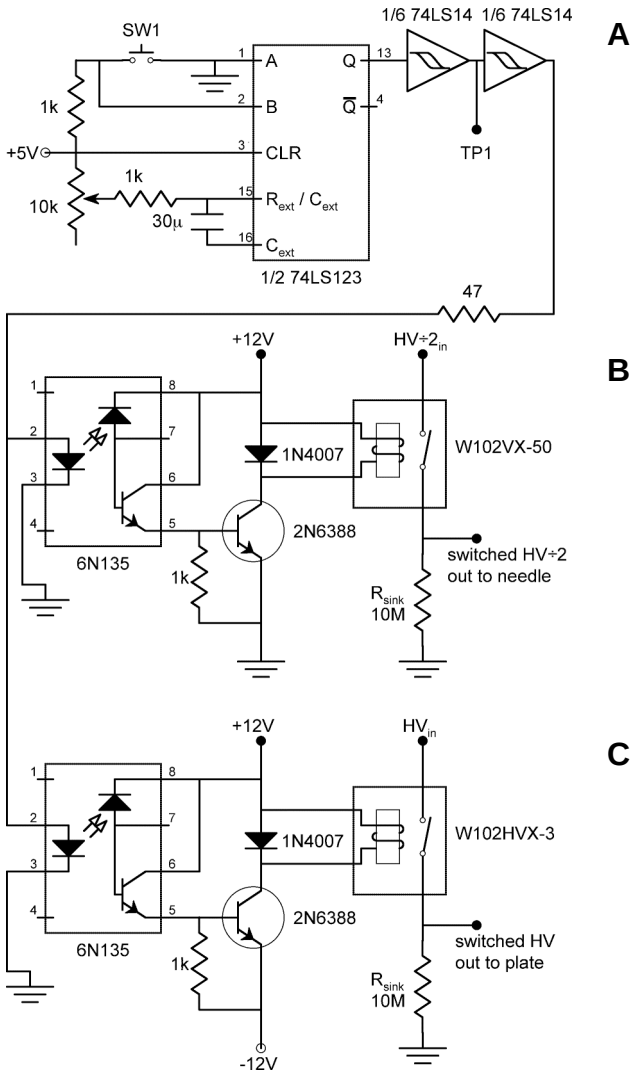

Figure A.2. Schematic diagram of the single droplet FIDI electronics. The FIDI pulse length is determined by capacitor C_{ext} and potentiometer R_{ext} in the 74LS123 monostable vibrator. When the switch (SW1) is closed, the generated pulse passes through two Schmitt triggers to provide a clean test point (TP1) for an oscilloscope and to provide enough current to drive the two high voltage switching circuits in frames B and C. Each switch consists of an optoisolator and a 2N6388 high power Darlington transistor. The switch in frame B provides the HV/2 voltage to the 28-gauge tube and the switch in frame C provides the HV to the parallel plate electrode.

HV switch in Figure A.2C is similar, however it employs a W102HVX-3 relay that may switch up to 10 kV because HV_{in} is typically between 7 and 9 kV. Because both relays are polarity agnostic, directing positive or negative progeny into the mass spectrometer does not require modifications to the circuit; only the polarity of the power supplies need be reversed. Each switch contains a sink resistance, R_{sink}, of 10 MΩ. This resistance is a four-by-four array of 10 MΩ, 1 Watt resistors where four sets of four parallel resistors are arranged in series. In this configuration, each resistor in the array is only sinking one quarter of the total voltage and one sixteenth of the total power. These arrays reduce the total load in accordance with the design limitations of the individual resistors.

A.3.3. LCQ mass spectrometer

A small number of modifications are required for the LCQ to successfully operate with the single droplet FIDI source. The commercial ESI or APCI head must be removed to accommodate the FIDI sled. Because the capillary inlet is grounded in the experiment, the wire delivering voltage to the body of the capillary is disconnected so that sparks and electrical discharges in the FIDI source are not directed through the capillary into the LCQ electronics. The capillary is well connected to an electrical ground.

FIDI acquisition occurs in the same manner as electrospray acquisition with few modifications. In the present design the activation and occurrence of FIDI is not coordinated with the LCQ acquisition. Therefore individual acquisition times are set to high values. Specifically the number of microscans is set to one, and the maximum inject time is set to 1000 ms (one second). The long acquisition time means the FIDI events need not be coordinated with the LCQ acquisition.

The remaining user-controlled instrument parameters are optimized using the same sample with the conventional ESI source. Parameters are tuned according to the built-in capabilities of the instrument.

A.4. Sample mass spectra

Figure A.3 shows the positive ion mass spectrum of α lactalbumin acquired with the single droplet FIDI source. The distribution of peaks is consistent with a multiply-charged protein and resembles an electrospray mass spectrum of the same solution under the same LCQ conditions.

A.5. Conclusions

Single droplet FIDI-MS opens new avenues in chemical and biological research. This chapter describes the single droplet FIDI source as well as design considerations and circuit schematics. Mass spectra from a single droplet of α lactalbumin are presented.

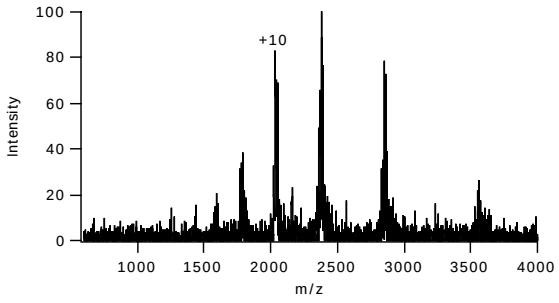

Figure A.3. FIDI-MS spectrum of 10 μm α lactalbumin in 50% water 50% acetonitrile. The distribution of peaks is indicative of a multiply-charged protein.

A.6. References

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