DEVELOPMENT OF
A COMPUTER-CONTROLLED
MULTIDIMENSIONAL LIQUID CHROMATOGRAPH
AND ISOLATED DROPLET INTERFACE

By

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ABSTRACT

DEVELOPMENT OF A COMPUTER-CONTROLLED MULTIDIMENSIONAL LIQUID CHROMATOGRAPH AND ISOLATED DROPLET INTERFACE

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This dissertation presents two instruments useful for the analysis of complex mixtures. The first is a computer-controlled liquid chromatograph incorporating two six-port, two-position valves for column switching. Column switching techniques have been used for increasing resolution, characterizing samples and simplifying chromatograms. The popularity of this technique has been limited, however, by the difficulty of reproducibly transferring eluent based solely on the time from injection. The multidimensional chromatograph presented here differs from previous designs in that valve control can be based on detector output as well as elapsed time. This method of eluent transfer is shown to be much more reproducible than that based solely on time. In addition, the instrument allows centralized control of all instrument functions using a unique operating system based on the FORTH computer language. This greatly simplifies the development of multidimensional chromatographic methods. Applications are shown for backflushing, heartcutting and mode switching.

Chromatograms of both model compounds and petroleum samples are shown.

The second instrument presented can assist in the interfacing of liquid streams to gas-based detection schemes such as atomic emission, atomic absorption or mass spectrometry. This instrument, called an isolated droplet generator, is based on the vibrating capillary principle of droplet production. It is capable of converting a liquid stream, such as that produced by an HPLC, to a sub-nanoliter sized monodispersed droplet stream generated at rates of up to 50 kHz. Selective charging and deflection can be used to select individual droplets or droplet packets from the main stream. This instrument is an improvement over older designs in that computer control imparts sufficient flexibility to make the device useful as a general-purpose high-resolution liquid handling system. In addition, droplet production parameters can be automatically altered to compensate for changing liquid streams, as in gradient liquid chromatography. Results are shown which indicate that droplet production is relatively unaffected by changes in viscosity and surface tension that typically accompany an HPLC gradient such as methanol to water. The effect of droplet introduction into a spark source is also discussed.

To Marguerite and Christopher,

my dearest friends

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I have often thought that the sociology of a research group would make an interesting subject for a dissertation. I wouldn't want to write it, of course, but it would be interesting nonetheless. During the five years or more that one spends doing research with fellow graduate students, many long-lasting relationships are developed. These relationships take many forms, but certainly teacher, student, mentor, friend and family are all adjectives which can be used to describe them. Graduate school has included some of the worst and some of the best times of my life; the people responsible certainly deserve some credit.

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PREFACE

This dissertation is primarily concerned with the design and implementation of two analytical instruments used in the support of work done under NASA grant number NAG 3-93. Among the goals of this grant were the development of methods for the determination and speciation of various metals in petroleum matrices. Several possible approaches were investigated using chromatographic separation followed by selective detection.

The first instrument is a computer-controlled multidimensional liquid chromatograph. The purpose of constructing this instrument was to investigate automated column-switching techniques for increasing the resolving power of liquid chromatography. In addition, such an instrument could provide more information for sample characterization.

The second instrument is an isolated droplet generator. This device was constructed to investigate the isolated droplet technique as a possible interface between various gas-phase detectors and the liquid chromatograph.

These two instrumental projects could have been separated and reported independently. Because the projects have a common origin, however, it was felt that a better approach would be to include both in the same body of text. To aid future researchers who may be interested in only one of the projects, each chapter of this dissertation has two main sections; the first pertaining to the multidimensional chromatography and the second to the isolated droplet generator. Every attempt has been made to distinguish these two sections clearly in each chapter. In addition, researchers interested in further applications of the multidimensional chromatography instrumentation should consult the 1985 Michigan State University doctoral dissertation of Marguerite R. Danna.

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I. INTRODUCTION

The term "multidimensional chromatography" was first used in conjuction with the practice of re-developing thin-layer chromatography (TLC) plates (1). After the end of the first separation, the plate is rotated 90° and developed a second time. If the solvent always travels in the vertical direction, the rotation of the plate changes the direction of solute travel on the plate and gives rise to a "multidimensional" thin-layer chromatogram. This technique can be employed to obtain additional resolving power based on simple physical separation as a result of turning the TLC plate. An additional enhancement in resolution can be obtained by varying the second mobile phase so that a different selectivity is produced for the second dimension.

When used in modern high performance liquid chromatography (HPLC), the term "multidimensional" generally refers to a collection of techniques involving switching a portion or portions of the effluent from one column onto a second column for further separation. Other common terms currently in use to describe this technique include column-switching, multiphase, multicolumn, or coupled-column chromatography (2). The use of the same or different stationary phases packed into the same column or connected in series is not considered multidimensional chromatography, since no switching mechanism is involved.

Several other related techniques which use similar instrumental setups are also commonly encountered in multidimensional chromatography. The most common of these are backflushing and trace enrichment. Backflushing refers to the practice of using a valve to reverse the mobile phase flow direction after some of the components have eluted from the column. Trace enrichment involves pumping a large quantity of sample through a column containing a stationary phase which strongly adsorbs the components of interest. The concentrated components are then eluted with a different solvent, and a portion is transferred to a second column for further separation.

Multidimensional chromatography can be carried out in an off-line or an on-line manner. Earlier approaches were usually of the former variety, where a portion of the first chromatogram was collected, evaporated, reconstituted, and finally, injected onto a second column. For convenience, automation, and better precision, on-line techniques are generally preferred.

Instrumentation for implementing on-line multidimensional HPLC (hereafter referred to as MHPLC) has generally involved multiple pumping systems and time-based event controllers. There are several drawbacks associated with such systems. First, the instrument cost is primarily dependent upon the number of pumps, since the pump is the most expensive component of an HPLC system. Thus, multiple-pump MHPLC may be prohibitively expensive for some laboratories or applications. Second, time-based valve switching is often analytically unreliable. Retention times in HPLC can vary considerably from sample to sample, making it necessary to reprogram the event controller. This is especially a problem with silica and alumina columns, where slight changes in water content drastically affect chromatographic behavior. Third, without centralized control of the experiment, such systems suffer from a lack of versatility in coordinating the actions of the various components.

This dissertation presents an MHPLC instrument which overcomes many of these difficulties. A single, intelligent solvent delivery system is described; this system can perform many of the functions which previously required multiple pumps. A central microcomputer controls and coordinates the entire experiment, including valve switching, data acquisition, and solvent delivery. Due to the added versatility of centralized control, intelligent valve switching can be performed based on the chromatogram itself. Valve switching can be based on the number of peaks detected, the percentage change in signal, the absolute signal level or the time after injection. A unique interactive operating system based on the FORTH language allows creative control procedures to be written. Such procedures can include repetition based on loop structures and conditional execution of events by using IF-ELSE-THEN constructions.

Multidimensional Detection in MHPLC

In the above discussion, the term "multidimensional" was introduced to describe a method of column-switching in chromatography. This term also has a much broader use, which refers to the nature of the data obtained from such an experiment. One example may be the coupling of a size-based separation with a polarity-based separation. The first dimension of information concerns the size of the species eluting from the first column. The second dimension, obtained when fractions are transferred to the second column, contains polarity information. When used in this sense, the term "multidimensional" can be extended to the detection process as well. If a diode-array UV detector were to be employed, the third dimension of data would contain information on the UV spectrum of the eluting components. Additional non-destructive detectors could be added in series to give additional dimensions of information. The potential for maximizing the information content from each chromatogram in this manner is a very active field of research.

One type of sample information useful to the chromatographer is a breakdown of the eluting components by element. Several researchers have used element-selective detectors to obtain empirical formulae of components eluting from a GC column (3), but interfacing liquid eluent to such detectors has been difficult. Nebulizers are commonly used to deliver liquids to flames, plasmas, and sparks for emmission-based detection. Most nebulizers, however, are inefficient and contain dead volumes too large for use in HPLC. In addition, there is often a high correlation between nebulization efficiency and liquid viscosity, which would severely limit the use of nebulizers in gradient HPLC where mobile phase viscosity changes dramatically.

In addition to the above MHPLC instrumentation, this dissertation presents an alternative to the nebulizer for introducing HPLC effluent into atomic excitation sources. The device is called an isolated droplet generator (IDG) and is based on principles similar to those used in some ink-jet printers. Liquid is forced through a capillary attached to a piece of piezoelectric material called a bimorph. The bimorph is vibrated, imparting longitudinal oscillations on the emerging jet. This causes the jet to break into reproducibly sized and shaped droplets. Selective charging and deflection is used to deliver the droplets to the excitation source.

Isolated droplet generators have been used in some form for nearly 35 years but this is the first instrument constructed that has been placed under complete microprocessor control. There are several advantages to this design. The droplet streams produced are very stable and reproducible. Droplet production parameters can be altered under computer control to respond to experimental needs or changing liquid composition. While the main focus will be on the use of the IDG as a possible HPLC interface, the instrument is versatile enough to function as a general purpose liquid handling device capable of manipulating liquids at the sub-nanoliter level.

II. HISTORICAL BACKGROUND

In this chapter the historical progression of published research which pertains to this dissertation is presented. Specifically, three reviews of the literature were performed. First, an extensive review of the major chromatographic journals has been assembled with respect to those references dealing with multidimensional liquid chromatography. Second, a somewhat less extensive but fairly representative review of literature dealing with element-specific detection is presented. The last section contains a comprehensive presentation of published research dealing with the development of isolated droplet generators.

Multidimensional HPLC

Multidimensional chromatographic techniques have been carried out for many years to assist in the separation of complex mixtures. Prior to the commercial development of modern HPLC instrumentation, thin-layer chromatography (TLC) was used extensively in this regard. To perform a multidimensional separation using TLC plates, the plate was rotated 90° and re-developed, usually with a different mobile phase. Since the advent of high-performance TLC plates, this technique has seen a minor resurgence in popularity. A good review of current multidimensional TLC applications has been written by Zakaria, Gonnord, and Guichon (4).

Modern column-switching techniques began appearing in the literature soon after the introduction of the first high-pressure, low dead-volume valve by Huber, Van Der Linden and Ecker (5) in 1973. A previous paper by Scott, Chilcote and Lee (6) in 1972 used a modified 6-port, 2-position injection valve to assist in the development of ion-exchange columns, but the time scale of the separation (14 hours) does not put it in the category of "modern" HPLC. The original concept of using valves to switch columns can be credited to the inventors of commercial water purification systems. Perhaps it was the success of these systems which prompted Snyder (7) and Liljamaa and Hallen (8) to suggest similar schemes for chromatographic applications.

Successful implementation of column-switching using high-performance columns required the minimization of extra-column band broadening resulting from dead volume in the valves and associated tubing. Dolphin and Willmott (9) addressed this problem in a paper in 1976, and concluded that the contribution of the valve to overall peak widths was not significant. Their theoretical evaluation compared well with the experimentally determined broadening contribution presented by Huber (5). Both authors found that long pieces of capillary tubing can cause significant broadening effects, however, and thus tubing lengths should be kept to a minimum.

As a complement to the band-broadening theory mentioned above, several papers dealing with the theoretical limits of resolution in multidimensional HPLC have been published. A recent report by Giddings (10) compares the separation power obtained from a variety of 2D separation techniques. The maximum separating power is obtained when the two separation mechanisms are totally independent. In this case, the predicted peak capacity is the square of the average capacity expected from a 1D separation. As the two separation mechanisms become more correlated, the peak capacity decreases. Snyder (11) reaches similar conclusions and also demonstrates several throughput advantages of 2D separation techniques. Guichon (12-13) has published several papers on this topic as well. Huber (14) has published a related paper which may be of interest on the application of information theory to multidimensional gas chromatography and low-resolution mass spectrometry. Another related paper explains the hysteresis effects observed when GC columns are backflushed (15). This paper is interesting in that it also explains why this phenomemon is not observed when backflushing HPLC columns.

Several research groups have been quite prolific in the field of multidimensional HPLC. Frei of the Free University of Amsterdam has published many papers, several in conjuction with Little and Stahel of Kontron Ltd. (16-18); Werkhoven-Goewie, also of the Free University (17,19-20); Erni, of Sandoz Ltd. (21-22); and Nielen and Brinkman, also of the Free University (23-25). Another researcher who has made several contributions to the literature is Majors of Varian Corporation(26-28). He has also collaborated with Apffel of Virginia Polytechnic (29), and Johnson and Gloor, also of Varian (30). Other prominent researchers include Dolphin and Willmott (9,31-32), Huber and Van Der Linden (5,33-34), Miller (35-36), and Harvey and Stearns (37-38).

Of the instrumental setups reviewed for column-switching, only one was found that used an on-line detector to trigger a valve change (32). The remainder used time-based sequencing. Approximately 50% employed two pumps (9,11,18-21,30,35,39,40-43), 31% used a single pump (5-6,29,32,37,44-46), and 19% used three or more pumps (16-17,47-49). Most systems employed a single valve (5-6,9,19,21,30,32-33,35,37,39,42-43,45-46,50), to perform either a simple heartcut (5,9,19,21,30,32-33,39,42-43,50), backflushing (37,35), or recycling (45). Of the remainder of the systems reviewed, 18% used two valves (11,29,41,44,47) and 25% used three or more valves for more complex switching arrangements (16-18,20,23,48-49). Eleven of the systems used two or more detectors (6,9,11,16-17,21,29,32,42-45); the remainder used only one.

Most applications of column-switching fall into the three categories of sample cleanup (17,20,43,46,51-52), trace enrichment (17,19,22,24-26,41,53), or class separation (54-56). The most common matrix was biological fluids, usually where the analyte was a pharmaceutical product (6,16-18,20,22,27-29,39,41-42,44,47,50,52,57). Other common matrices were foodstuffs (16-17,21,27,30-31,33,40,44,46), petroleum (35,37,54-56,58-60), and aqueous waste streams (17,19,23-24). Table 1 summarizes applications from some selected references. In addition, several reviews and general discussions of multidimensional HPLC have also been published (16,26-28,35,61-63).

Element Specific Detection

The problem of positive identification has always existed in chromatography. Since the separating power of a column is limited, one always runs the risk of mistaking the identity of a peak for another compound having a very similar retention time. This is true even for the best case, where the peak identity is known and a standard exists for comparison. For those cases where an unknown sample having many components is separated, identification is tedious at best and impossible at worst.

Table 1. Selected multidimensional applications.

Sample type	Primary Mode	Secondary Mode	Reference
Pesticides in milk	LSC	LSC	31
Herbicides in cereals	n-BPC	n-BPC	33
Drug metabolites in plasma and urine	RPC	RPC	44
Acids in wine	RPC	RPC	17
Chlorophenols in water	RPC	RPC	17
Malathion in tomatoes	GPC	RPC	30
Limonin in grapefruit peels	GPC	RPC	30
Additives in rubber stocks	GPC	RPC	30
Vitamins in food protein supplement	GFC	RPC	27
Antibiotics in serum	IEC	RPC	35
Sugars in candy	GFC	RPC	29

Ettre (64) has put the problem in perspective with a simple calculation. Assuming a 50-m long glass open tubular capillary column with a HETP of 0.33 mm for n-hexadecane, the separation number for the pair of normal paraffins C_1 and C_{16} would be 97. This means that about 100 peaks can be separated in the time interval between the two paraffins, in this case about 20 minutes. Although this is a remarkable performance, the number of possible compounds that could elute within this interval is probably at least ten times this amount.

Fortunately, for industrial samples, the number of species is limited by the chemical process itself. However, for natural or biological samples this is not true and some other means must be used for peak identification. Substance-selective detectors can play an invaluable role in this regard.

Selective detection is not new to chromatography. The term "chromatography" itself implies detection by color -- a fact which Tswett (65) emphasized in his first paper. Before going further, a point of distinction should be made between "selective" detection and "specific" detection. Specific detection refers to a process that only gives a response to a single compound. Selective detection, on the other hand, gives a response to a class of compounds. Egan (66) presents a good discussion of this distinction. Depending upon the point of interest, sometimes a detector can be either selective or specific. For example, an atomic absorption detector is specific for a given element, but selective for the class of compounds containing that element.

The first limited-response detector was, in fact, a specific detector. In the late 1950's, Bayer and Anders (67) extracted the glands of nine female silk moths and injected the extract into a GC. At the end of the GC column, they placed a male silk moth in a small box. A number of peaks were obtained and most of the time the male moth remained motionless in the corner of the box. But when one particular peak eluted, he started to wiggle his wings and run around, becoming very excited. This particular peak, of course, corresponded to the pheromone of the female silk moth.

Selective detectors for liquid chromatography first appeared around 1933 and were based on UV spectroscopy (64). Today, most LC analyses are done with the help of selective detectors. Commercial detectors are available based on UV or visible absorbance, electrochemical redox reactions, post-column chemical reaction, and fluorescence. Several reviews have been published on the current state of detector technology for HPLC (64,68-70).

In contrast to the development of selective GC detectors, which were designed specifically for this purpose, most selective LC detectors actually represent existing analytical instruments modified to permit the direct coupling of the column effluent. Thus, the ability of the interface to deliver the column effluent efficiently and with a minimum of band broadening often determines the success of the detector. This is especially true of instruments where the detection process occurs in the gas phase.

Most of the research concerning element-specific detectors for liquid chromatography has been centered around mass spectrometry or atomic spectroscopy. Of these two techniques, the classification of a mass spectrometer as a "detector" for LC may be somewhat of a misnomer. It is probably more appropriate to call the less expensive LC an

introduction technique for the mass spectrometer. The interfacing requirements of the two systems are quite different, since most mass spectrometry takes place at very low pressures. Nevertheless, some interesting work has been published concerning LC/MS interfacing techniques (71-73).

Atomic spectroscopy has been interfaced with HPLC by a variety of researchers. Van Loon (74) has published an excellent review on this topic. Of the instrumental innovations noted, most dealt with novel ways of interfacing the HPLC effluent with the instrumentation used for atomic spectroscopy. Van Loon notes that HPLC is incompatible with most AAS nebulizers and with the stepwise operation of commercial electrothermal atomizers (74). The liquid flow rate used in HPLC is not compatible with most concentric nebulizers, but is often compatible with crossed-flow nebulizers used in many inductively coupled and DC plasma atomizers. Many approaches have been used in designing an interface with the following typifying some of the more common.

Brinckman et al. (75) used a carousel-type automatic sampler to collect fractions for subsequent analysis by furnace AAS. The advantages of this approach are said to be better detection limits and small sample size. A modification of this approach was used by Vickrey et al. (76) where a UV detector is used to identify the peak of interest and the entire peak is collected for subsequent analysis by a furnace AAS unit.

Because the flow rate typically used in HPLC is too slow (less than 2 mL min^{-1}) to be interfaced directly to the nebulizer of an AAS burner, Slavin and Schmidt (77) used a discrete injection technique. In this approach, the effluent from the HPLC column is allowed to drip into a teflon funnel which is connected to the inlet of an AAS nebulizer. Each drop is then atomized separately, and gives a chromatogram comprised of a series of spikes. The authors claim that 0.1 mL droplets are sufficient to give a steady state AAS signal; thus the technique retains the full sensitivity of continuous AAS. Further investigations of this technique can be found in the "Evaluation and Applications" chapter of this dissertation.

Several different nebulizers have been evaluated in terms of dead volume and efficiency by Hausler and Taylor (78). They found that a spray chamber utilizing a drain that exited on the same side as the nebulizer decreased peak broadening by allowing a rapid and clean drain. A spray chamber of this type is now commercially available from Applied Research Laboratories (ARL). Another interesting nebulizer design was published by Lawrence et al. (79). In this design, a microconcentric nebulizer is mounted directly under the plasma, thus avoiding dead volume associated with a spray chamber altogether. While peak broadening is minimized, the nebulizer was not operated above flow rates of 0.2 mL min^{-1}. Problems with plasma instability were also noted.

Solvents are often a problem in LC using atomic spectroscopy detectors, especially when plasmas are used as the excitation source. Many applications that use reversed-phase or ion-exchange solvents, where a major component of the mobile phase is water, have been published (80-83). Nonaqueous mobile phases, however, can cause instability with plasmas and carbon buildup with both plasmas and flames (83). Microbore HPLC is an alternative which may relieve some of the solvent effects, but there appears to be a loss in sensitivity due to the smaller sample sizes associated with these columns (84).

Isolated Droplet Generators

From the preceding discussion it is obvious that a major limitation in utilizing LC-AAS or LC-AES is the lack of an efficient, low dead-volume interface. One device which may prove useful in this regard is the isolated droplet generator. These devices can be made with very low dead volume and the amount of sample transferred can be regulated to suit the excitation source.

The discovery of the isolated droplet production phenomenon is usually attributed to Lord Rayleigh (85-86) in 1878. While these papers were the first attempt to explain the theory of droplet formation, the first experimental observation of droplet production was reported by Savart (87-88) in 1833. Both of these authors reported conditions under which a liquid jet breaks apart in a uniform manner.

Since the first reports of droplet production, many different types of droplet generators have been reported. In 1947 Lane (89) constructed an apparatus in which a droplet was blown off the tip of a capillary by a jet of air when the droplet was of the desired diameter. Mason and Brownscombe (90) constructed a similar apparatus in 1964 which could also produce charged droplets. Cheng and Cross (91) also constructed an air-actuated droplet generator in which a concentric stream of air controlled the velocity of the droplet.

Another type of droplet generator which several researchers have constructed is based on a vibrating reed principle. The reed, or stylus, is dipped into a reservoir where a small amount of liquid clings to its surface. Upon withdrawal, a filament of liquid is drawn out of the reservoir. The filament then collapses upon itself, forming a droplet, which is allowed to fall under the action of gravity. The first droplet generator of this type was reported by Wolf (92) in 1961. While the fastest droplet production rate was only 120 Hz, the droplet size could be varied between 10 and 50 microns by adjusting the penetration depth, diameter, and construction material of the stylus. Abbott and Cannon (93) and Shabushnig and Hieftje (94) have also constructed droplet generators of this type. A disadvantage of this type appears to be the large dead volume associated with the solvent reservoir and the inability of the stylus to draw filaments from solutions with low wettability characteristics (nonaqueous).

The third, and most common, type of droplet generator is based upon the induced breakup of a liquid jet. These droplet generators are most closely related to those originally described by Rayleigh (85-86). The first was described in 1964 and in subsequent papers by Schneider et al. (95-96). This type is based on a capillary vibrated by a piezoelectric crystal. The vibrations are transferred to the emerging jet and cause it to break up into droplets under the action of surface tension. The size of the droplets is determined by the wavelength of the disturbance launched onto the surface of the jet. Lindblad and Schneider (97) published an improved version which employed a different mounting system for the capillary. All three versions were capable of producing charged droplets which could then be deflected into a trap when not needed.

Hendricks and Schneider (98) published a paper which deals with the theory of droplet stability. This work is basically a detailed re-derivation of Rayleigh's equations using modern notation. Hieftje and coworkers (99-105) produced several versions of droplet generators for application in analytical chemistry. Bastiaans and Hieftje (99) used the droplet generator in flame spectroscopy to perform high precision "null point" measurements. Hieftje and Malmstadt (100-101) also used droplet generation to study fundamental flame spectrometric processes. Hieftje and Mandarano (102) and Lemke and Hieftje (103) used droplet generators as a basis for an automated titrator and a pH-stat, respectively. Steele and Hieftje (105) constructed a micro-titrator based on this method of liquid introduction.

An updated controller for this type of droplet generator was presented by Russo et al. (104); it uses a crystal oscillator-based frequency source for enhanced stability. Further advances in control circuitry have been presented by Seymour and Boss (106). The "Instrumentation" chapter of this thesis also contains a description of the author's control circuitry, which allows complete computer control of droplet production parameters.

Two other variations of the jet-type droplet generator have also been published. The first variation uses a vibrating orifice instead of a vibrating capillary. This type was used by Joshi and Sacks (107) in a circular slot burner design. Some ink-jet printers use a similar vibrating orifice design (108) and TSI, Inc. of St. Paul, Minnesota manufactures a commercial instrument of this type for controlled aerosol production.

The second variation was published by Willoughby and Browner (73) as the "MAGIC" interface for LC-MS. In this design, which uses a capillary jet, the vibrations are not artificially imposed. Natural background frequencies already present on the jet are allowed to cause jet disruption and an air stream separates the droplets before they have time to coalesce. Droplet size is varied by changing the diameter of the jet.

III. INSTRUMENTATION

In this chapter, the instrumentation pertinent to the research in this dissertation is presented. The first section deals with the multidimensional liquid chromatograph. This instrument contains some modules which are commercially available; these are described to the extent that is necessary for comprehension of their function within the instrument. The other modules which have been designed in-house are presented in more detail. The second section discusses the isolated droplet generator. This instrument has been completely designed in-house and is presented here in two phases. The first phase in its development was an implementation as a stand-alone system with independent control circuitry. In the second phase, the instrument was placed under computer control, making it much more versatile and easy to use. Both phases are discussed in detail.

A. Multidimensional HPLC

1. System Overview

Any HPLC system consists of four basic components: a solvent delivery system, a column to perform the separation, a detector, and a readout/recording device. A multidimensional HPLC system has two additional components: valving to alter the chromatographic flow path and a controller to properly sequence the experiment.

The simplest control device for sequencing multidimensional chromatography is a chemist with a stopwatch and a quick hand. Due to the tedium of this approach, however, an automated controller is highly desirable. Most controllers used in the past have been time-based event sequencers equipped with mechanical relays which can then be used to trigger valve changes or start and stop a pump. While this approach is very helpful, developing a reproducible time-based method is difficult. The main reason for this is that HPLC retention times can vary significantly from one injection to another. A much better approach, which is implemented in the system described herein, is to base the events on the chromatogram itself, as well as time. This has been accomplished by digitizing the detector signal and utilizing a microcomputer to analyze the data in real time.

A second consideration in constructing the multidimensional HPLC system was cost minimization. Previous designs delivered each solvent mixture with a separate pump and switching valve. These components add a great deal of expense to the system. An alternative is to mix the solvents prior to the high-pressure delivery stage with a single proportioning valve and deliver the resulting mixture with a single pump. Intelligent, microprocessor-controlled solvent delivery systems of this design are capable of gradient operation, solvent selection, flow rate programming and other advanced features needed in MHPLC. Since the main cost of a solvent delivery system is the hardware, one intelligent pump can be purchased for much less than several "dumb" pumps of comparable quality.

A third consideration in constructing this multidimensional HPLC was to provide full integration of all components of the instrument. This was accomplished by using an 8085 microcomputer as a centralized controller. Figure 1 illustrates how the microcomputer operates in the laboratory computing environment. Although this is a hierarchical computing environment, the microcomputer is actually the controller for the entire experiment, issuing commands to both lower and higher levels of the hierarchy. With intelligent centralized control, the term "event" takes on a much broader meaning than a simple valve switch. An experimental event could be a change in the data taking

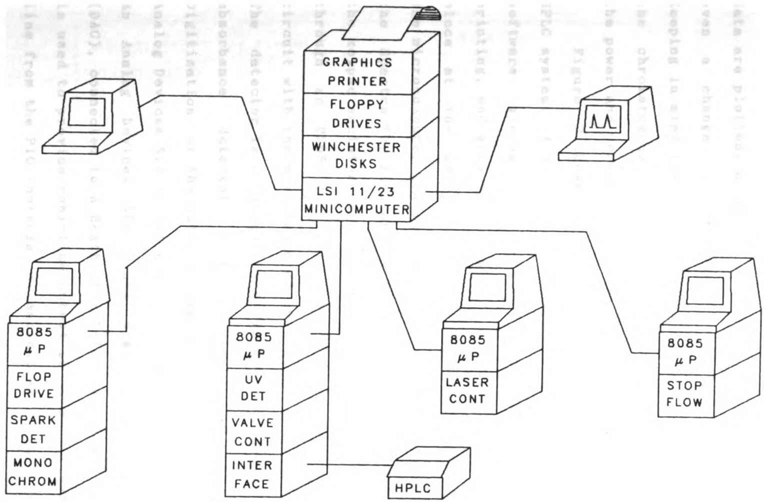


Figure 1. A hierarchical computing environment.

rate, a change in the scale factor or offset with which the data are plotted, a solvent change, a flow rate change or even a change in the chart recorder status (on/off). Keeping in mind that any of these events could be based on the chromatogram itself as well as time, one can appreciate the power and versatility of this method of control.

Figure 2 shows a block diagram of the multidimensional HPLC system. A DEC 11/23 minicomputer is used for data and software storage, high-level numerical manipulation, printing, and graphics output. Experiment control takes place at the level of the Intel 8085-based microcomputer. The microcomputer issues commands to the microprocessor in the Spectra-Physics SP8700 solvent delivery system through the keypad emulator circuit. Valve switching is implemented through an 8255 parallel input/output (PIO) integrated circuit with the assistance of the valve control circuitry. The detector is a Chromatronix fixed-wavelength ultraviolet absorbance detector with a low-volume flow cell. Digitization of the detector signal is accomplished with an Analog Devices 574 12-bit analog-to-digital converter (ADC). An Analog Devices 558 8-bit digital-to-analog converter (DAC), connected to a Heath EU-205-11 strip chart recorder, is used to provide real-time output of detector response. A line from the PIO controls a mechanical relay which can start or stop the chart drive. All timing operations are performed by an Advanced Micro Devices AM9513 system timing

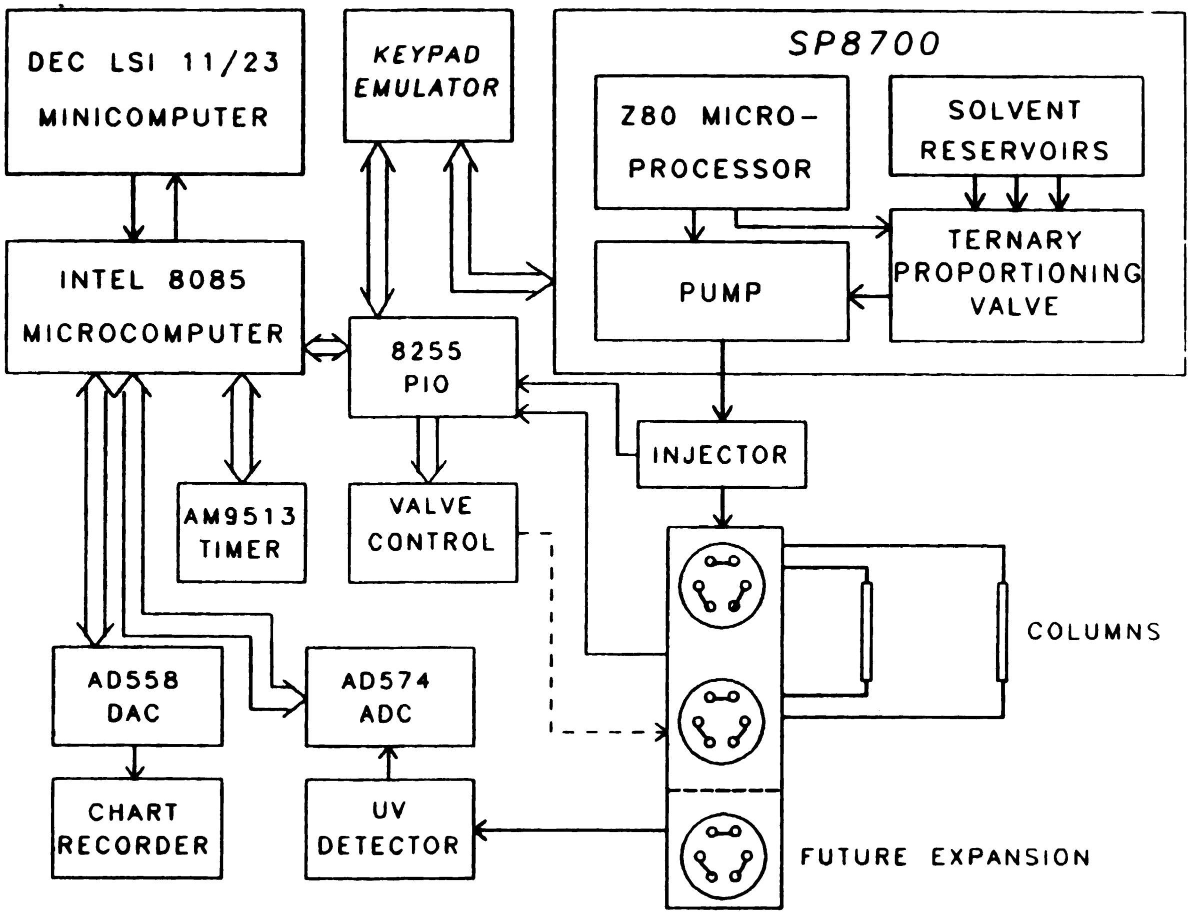


Figure 2. Block diagram of the multidimensional HPLC system.

controller chip operated at 1 MHz by an on-board crystal oscillator. The valves are 6-port, 2-position low dead-volume Rheodyne valves capable of withstanding 6000 psi. Each of these components is discussed in more detail below.

2. The Microcomputer System

The microcomputer system is based on the Intel 8085 microprocessor and uses a bus design developed by Newcome and Enke (109). Table 2 lists the component boards and their characteristics.

Table 2. Microcomputer components.

Component	Characteristics
8085 CPU board	6 MHz oscillator, 6 status LED's, reset & halt switches
RAM/ROM board 1	10K ROM based in 2716 IC's, 6K RAM based in 2016 IC's
RAM/ROM board 2	16K RAM in eight 2016 IC's
8259 Interrupt controller	8 vectored maskable interrupts, 4 used for I/O, 4 unused
8251 Dual USART	USART 1 - 11/23, USART 2 - term., 4800 baud for both
Chip select 1&2	512 byte range, 8 64-byte unqual. & 8 8-byte read/write qualified
Terminator	Active bus termination board
AM9513	5 programmable 16-bit timers, 1MHz in
8255 PIO	3 8-bit programmable I/O ports
AD574 ADC	12-bit A/D, sample & hold, 0.025 μs conversion time, -10 volt to +10 volt
AD558 dual DAC	2 8-bit DAC's 0-10 volt or 0-2.56 volt

This microprocessor design is a twin-bus modular system which can be custom tailored to meet a wide variety of laboratory automation needs (109). The particular implementation used here contains the active components listed above mounted on two motherboards, which plug into a backplane; the latter can contain up to four motherboards. All input and output between the microcomputer and the world is routed through the backplane. The system has an 8-bit data bus and a 16-bit address bus which can support up to 64K bytes of address space. System routines for communication, editing, and compilation of user programs written in FORTH are stored in 10K bytes of read-only memory (ROM). The compiled programs are stored in 22K bytes of random access memory (RAM), along with user variables, system variables, and communication buffers.

Communication to the terminal and the 11/23 minicomputer takes place over RS-232 serial lines via two universal synchronous/asynchronous receiver transmitter (USART) integrated circuits, which are both hardwired to operate at 4800 baud. Communication is interrupt-driven, with the USARTs requesting interrupts through the 8259 interrupt controller.

Each motherboard is equipped with a chip select module which decodes the signals present on the address bus. This module divides a 512-byte range of addresses into eight 64-byte segments. The base address is hardwired on the chip select board. The highest segment is further subdivided into eight, eight-byte segments which generate read/write qualified chip selects. When any location in each of these 64-byte segments is accessed, a corresponding active-low pulse is generated for as long as the address remains valid. For the read/write qualified segments, the pulse is generated only during the interval when the address is present and a read or write is valid. The chip selects generated are hardwired to the individual modules by wire wrap connection. By this approach, a single module can generate signals to map the entire motherboard into the system's memory, which avoids duplication of circuitry. Each module uses a chip select, with the exception of the CPU, which is always active, and the RAM/ROM boards, which generate their own.

Several peripheral modules are also used in the system. The first, a parallel input/output module based on the 8255 has been discussed elsewhere (109) and shall only be briefly described here. This device has three independent, programmable eight-bit ports. Ports A and B can be selected to be entirely for input or entirely for output. Port C can be programmed the same as A and B, or it can be split, with four bits used for input and four used for output. As implemented in this system, port A is used to communicate with the SP8700 solvent delivery system. Six lines are used to specify a keystroke, a seventh line is used to operate the keypad emulator interface, and the eighth is not used. Port C is used in the split mode. Four output bits control valve switching, and four input bits sense valve position. Port B is unused. The exact connections to this chip are shown in Figure 3, and discussed in more detail in the section on valve control.

Two eight-bit AD558 DACs are installed in the system. These devices interface directly to the data bus and are hardwire selectable to provide either a zero to ten volt range or a zero to 2.56 volt range. Only one of the DACs is used. It is configured to operate in the ten volt mode and is used to drive a Heath EU-205-11 strip chart recorder. To facilitate direct connection of the DAC to the one volt full scale input on the recorder, a voltage divider was placed on the output of the DAC. This divider is composed of a fixed 1/4 watt 8.6 kΩ resistor in series with a 1 kΩ potentiometer, which is adjusted to give one volt full scale at the junction of the two resistors. This approach is preferable to using only part of the 2.56 V range because the full eight bits of resolution are preserved.

Digitization of analog signals is accomplished with the AD574 12-bit ADC. This module also contains a sample and hold based on the AD583 or its pin-compatible equivalent the SHM-IC-1 (Datel-Intersil, Mansfield, MA). Conversion is initiated by writing to the address programmed on the chip select board. The resulting low pulse is sent to the chip

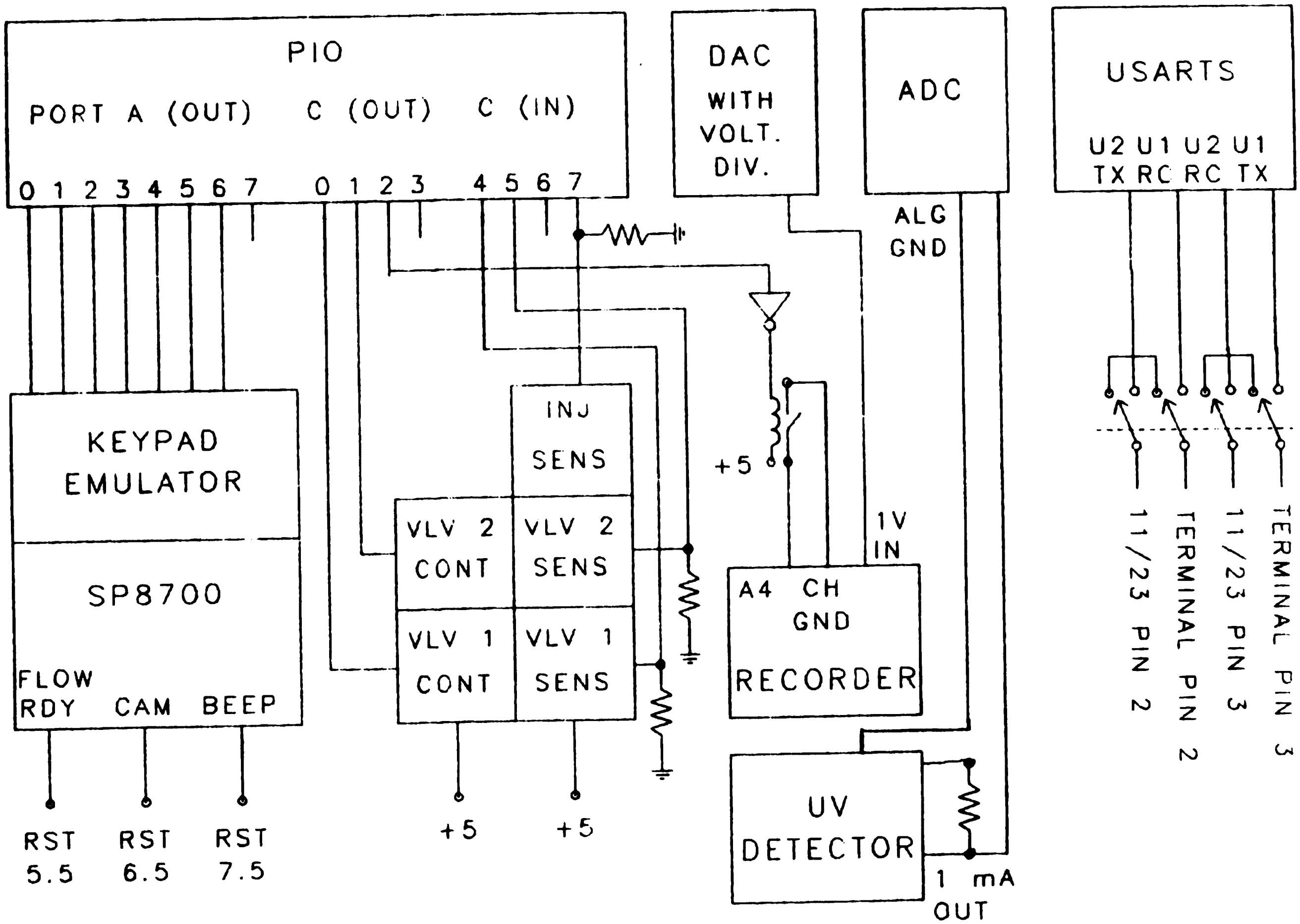


Figure 3. Microcomputer system wiring diagram.

enable pin. When the active-low write pulse reaches the read/not convert pin, conversion begins. During conversion the ADC status line goes high. This line is tied to the sample and hold to freeze the analog signal until the conversion is complete. This line may also be polled by the microprocessor by reading from the memory space corresponding to a second chip select used by this board. This board uses a separate analog ground to help minimize the bus noise associated with digital ground. Normal noise fluctuations are $\pm$ 3 ADC units. This noise level is acceptable for present applications, but could possibly be improved if the status line is used to halt the CPU during the conversion cycle. More information concerning this module is also available (110).

System Timing Controller

Timing and waveform generation functions are provided by the Advanced Micro Devices AM9513 System Timing Controller (STC). Because this peripheral is referred to extensively in the following discussions, a brief description of the device is given here.

The STC contains five independent, programmable 16-bit counters. This extremely versatile device can be programmed under software control to perform operations such as variable modulus up/down counting, timing, frequency generation, pulse generation and others. The main

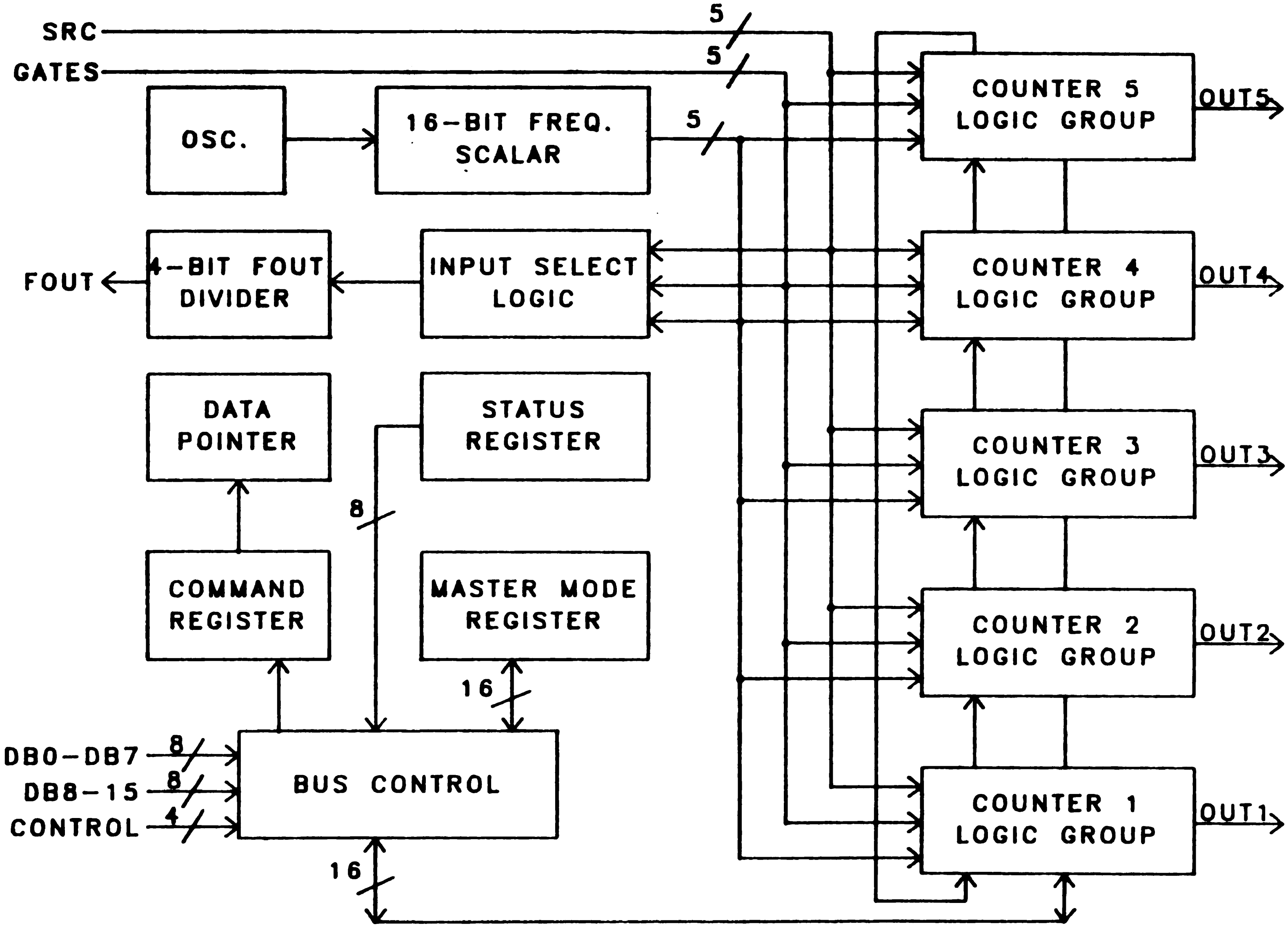


Figure 4. Block diagram of the AM9513 system timing controller.

components of the IC are shown in Figure 4. With the exception of the oscillator, all counters, scalars, registers and logic are contained in the device. Provision has been made for direct connection to the data bus, with self-contained bus control and communication logic. As implemented in our system, however, a 74LS245 tri-state buffer was placed between the AM9513 and the data bus. Inputs and outputs to the individual counters were also buffered for added protection. A 1.000 MHz Hytek HY-4550 oscillator is used as the source frequency.

The STC uses a single chip-select line in conjunction with the read, write, and A_0 address lines. The STC is addressed by the external system as two locations: a control port and a data port. Address line A_0 is used to select between the two. The control port provides direct access to the status, command and data pointer registers. The data port is used to access all other internally addressable locations. The data pointer register is used to control the data port addressing. In this manner, complete control of the device is possible using simple read/write operations.

Options which pertain to the chip as a whole are controlled through the master mode register. The system options are listed in Table 3. In addition to such control parameters as bus width and data pointer sequencing, the master mode register controls a 16-bit prescalar and a 4-bit output divider. The prescalar generates five frequencies for counter input. These frequencies include the original oscillator frequency and four subdivisions. The divisions can be in powers of ten or powers of 16. The master mode register also controls a 4-bit gated counter which can present a pulse stream to the FOUT pin.

Table 3. AM9513 master mode configuration options.

FUNCTION	OPTIONS
Frequency scalar	Binary or BCD division
Data pointer control	Enable/disable autoincrement
Data bus width	8-bit or 16-bit data bus
FOUT gate	Enable/disable FOUT output
FOUT divider	Divide by 1,2, ... 16
FOUT source	F1, F1/10, ... F1/10000
Time-of-day control	Disable/enable, source freq.

Frequencies for the counters and the FOUT divider are selected from a 15-source internal frequency bus. Ten of the 15 sources are derived from individual pins. The other five are derived from the 16-bit prescalar. Any source on the frequency bus is available to any or all of the counters. In addition, a counter can select the output of the next lowest counter as its frequency source. In this manner, individual counters can be concatenated.

A block diagram of a counter logic group is shown in Figure 5. Each counter logic group consists of a counter, a mode register, a load register, a hold register, and an

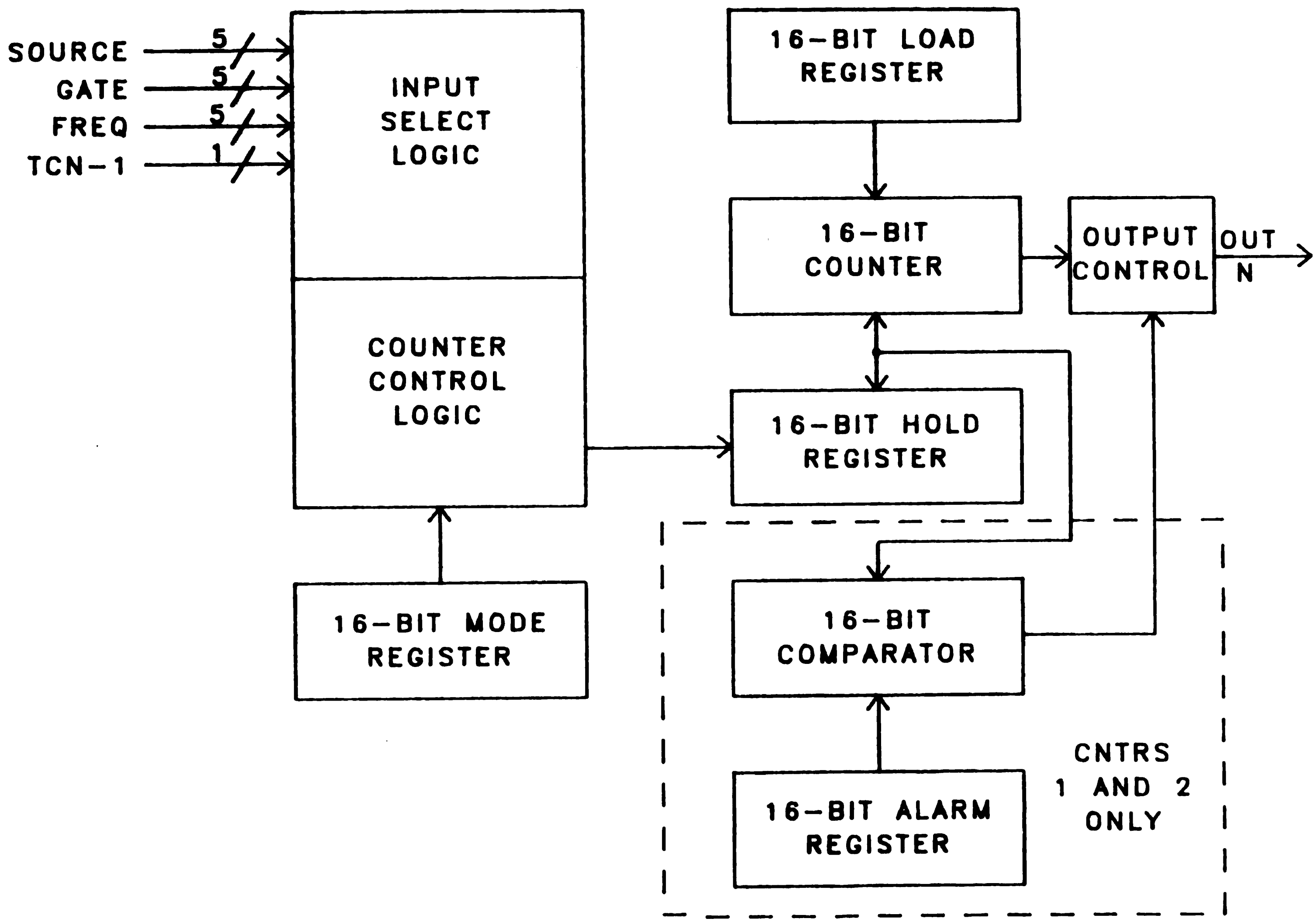


Figure 5. Block diagram of an AM9513 counter logic group.

output control. The mode register sets the configuration of the counter. The options available for each counter are shown in Table 4. The load register contains a value to be used as the initial count. The hold register can function either as an alternate load register, or it can be used to save the current value of the counter without interrupting the count process. The output control is basically an optional flip-flop. When enabled, the output state is inverted at each terminal count. This toggled-output option can be used to generate variable duty cycle pulse trains. When the output control is disabled, a pulse is generated at each terminal count. Active-high or active-low pulses can be selected. A variety of gating options is also possible for each counter. Several other good discussions of this device are available (111-113).

In the multidimensional HPLC instrumentation, all five counters of the STC are used for timing and delay generation. Counter 1 is used to generate a delay for data taking. Counter 2 can be used in user programs to generate short delays. Counters 3 and 4 are used to count the number of tenths of seconds from the start of the chromatogram. Counter 5 generates a delay for real-time peak recognition. These counters are redefined for the isolated droplet generator instrumentation, as discussed below.

Table 4. AM9513 counter mode register options.

FUNCTION	OPTIONS
Gate control	Disable/enable
Gate input (edge)	Rising/falling
Gate input (level)	High/low
Count source selection	
Count edge	Rising/falling
Count source	Source/gate/internal
Count control	
Reload register	Load/load and hold
Repetitive count	Enable/disable
Count mode	Binary/BCD count
Direction	Up/down
Output Control	
Inactive	High impedance/low level
Active	High/low terminal count or toggle output state

3. Solvent Delivery Requirements

Any solvent delivery system for modern HPLC must meet the requirements of providing a constant, pulse-free flow of both aqueous and nonaqueous solvents at high pressure (1000-5000 psi). When the solvent delivery system is used for multidimensional work, several additional restrictions are imposed. First, because switching a column into or out of line is usually accompanied by a large change in backpressure, the pumping apparatus must be able to respond to this change in a reasonable period of time and maintain the desired flow rate. If one could guarantee that the column would always have the same backpressure, this restriction could be loosened. For example, if the flow rate decreased to a lower, but reproducible level, the separation could continue at the new flow rate. The new rate would probably be below the optimum, however, so at best a compromise would have to be made between the two states of the system. In reality, a column does not contribute a reproducible backpressure. Factors such as mobile phase viscosity, temperature, condition of inlet and outlet frits, and general aging all contribute to changes in resistance to solvent flow. Therefore a pump which can provide constant flow regardless of pressure is highly desirable.

Another requirement imposed by multidimensional chromatography is the ability of the pump to deliver multiple solvents, either in a step-gradient fashion or preferably as a continuous gradient. Most applications of single-column chromatography can be performed as isocratic separations, with the exception of extremely complex samples. With multidimensional separations, on the other hand, solvent changeover and gradient elution are the rule rather than the exception.

A third requirement for an automated multidimensional HPLC system is a provision for the solvent changeovers, gradients, flow rate changes, and so on to occur synchronously with other events in the experiment. This requirement implies some sort of communication link between the experiment controller and the solvent delivery system, or at the very least the ability to synchronize the solvent delivery controller with the device controlling the rest of the experiment.

The first two requirements were met satisfactorily by a commercial solvent delivery system, the Spectra-Physics SP8700, which is discussed below. To meet the third requirement, it was necessary to construct an interface between the microcomputer and the SP8700. This interface is based on a keypad emulation principle and will be presented in detail.

Description of the SP8700 Solvent Delivery System

Figure 6 contains a block diagram of the SP8700 solvent delivery system. The solvent flow path begins from three glass solvent reservoirs. These reservoirs are equipped with sintered metal aerators which are used for helium degassing of the solvents. From the reservoirs, the solvents are individually routed to their respective intake ports on the ternary proportioning valve. The ternary porportioning valve, under microprocessor control, mixes the degassed solvents in the volume percentage specified by the operator. The mixed solvent then enters the pump through an inlet check valve. The pump motor, under control of the CPU and two feedback loops, is caused to vary in speed versus time and thus control the solvent flow rate. The first feedback loop monitors the pressure transducer at the pump outlet and feeds its signal into a differential amplifier

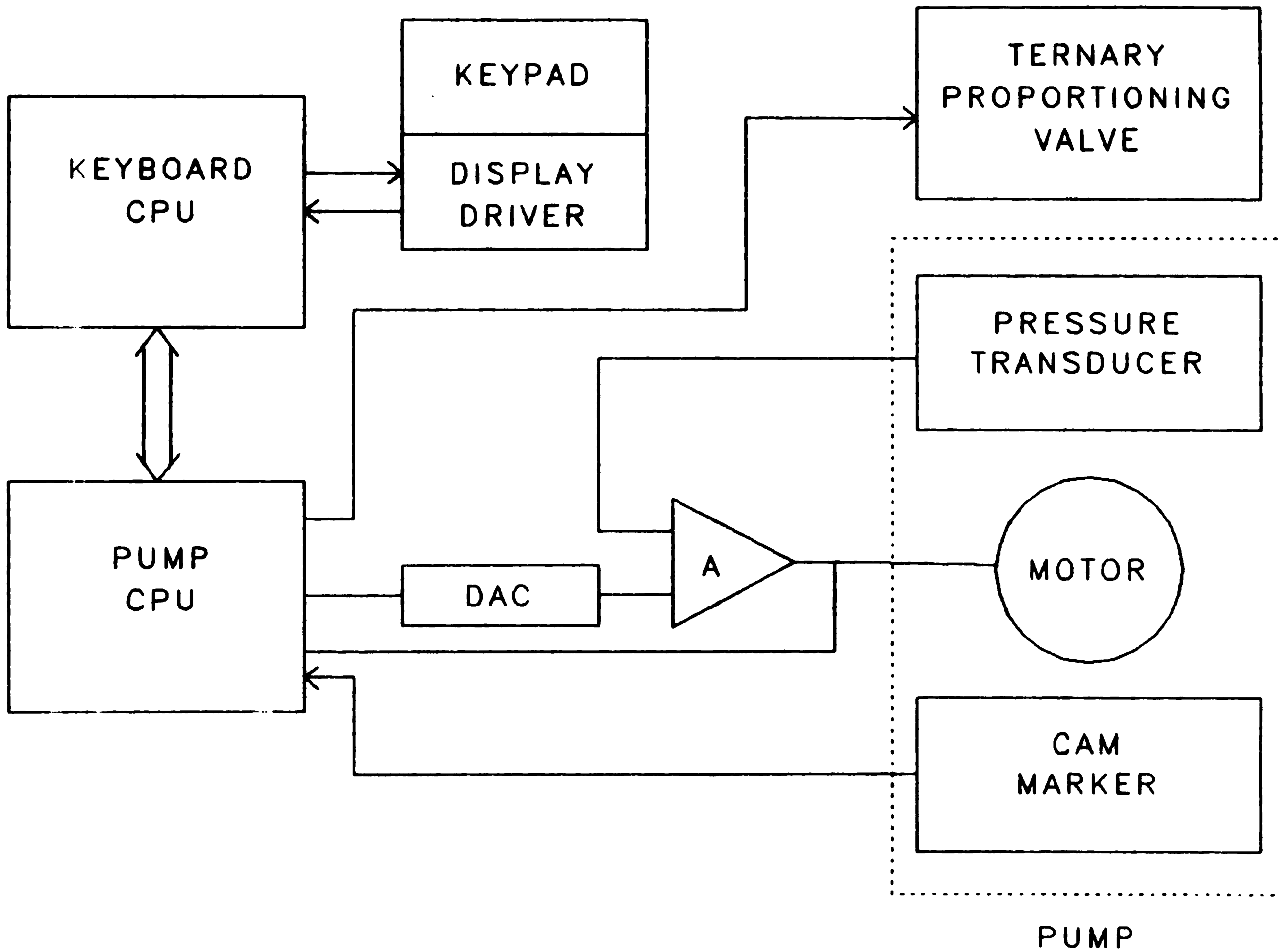


Figure 6. Block diagram of the SP8700 solvent delivery system.

whose output is connected to the pump motor drive circuitry. This loop is used to provide short-term pressure control. The second loop is a digital feedback loop which monitors the number of motor steps and the cam marker. The pump progress is checked ten times each cam cycle by measuring the time required to complete a fixed number of motor steps. The difference between the measured time and a reference time is used to generate a new target pressure which is then sent through a DAC to the other input of the differential amplifier in the first feedback loop. The difference between the new target pressure and the actual pressure sensed by the pressure transducer produces a signal which controls the pump motor speed. Thus, the first loop provides short-term pressure control, while the second loop provides long-term flow control.

After leaving the pump through an outlet check valve, the solvents pass through a static mixer to a column bypass valve. This valve is used for priming the pump or clearing the mixing chamber of old solvent. The static mixer is simply a 10 cm column packed with glass beads. A silica pre-saturator column has been used in place of the static mixer with no apparent change in pump performance.

Solvent delivery parameters can be entered manually through an 11 x 4 keypad array on the front of the instrument, or automatically through the keypad emulator described below. The memory of the SP8700 is organized into ten parameter files containing seven entries each. These files provide some limited programming capability which is usually adequate for simple, single-column operation. For multidimensional chromatography, however, seven entries are not sufficient to completely describe a run, and there is no provision for automatically linking one file to the next. There is also no provision for permanent storage of parameter files. For these reasons, and others discussed below, a keypad emulator was designed to interface the SP8700 to the 8085 microcomputer.

The Keypad Emulator Interface

The addition of a means to allow the 8085 microcomputer to control the SP8700 increased the capabilities of the system in several ways. A larger number of parameter changes can now be entered, and parameter files can be automatically linked. The parameters can be stored permanently, on hard disk, floppy disk, or as hard copy; disk-stored parameters can be retrieved and re-entered at will. The SP8700 clock and the microcomputer clock can now be synchronized so the entire experiment takes place in the same time frame. Finally, since the microcomputer is sensing the detector signal as well as controlling the SP8700, a feedback loop can be established so that the parameters of the HPLC experiment can be altered on the basis of the chromatogram itself.

Keypad emulation is a general method of control applicable to microprocessor-based instruments that use "polled" keypad arrays (114). The method uses an integrated circuit (IC) multiplexer and an IC demultiplexer to simulate a keystroke by connecting the desired column of the array to the desired row under computer control. Keypad emulation utilizes fully the existing instrument circuitry, and allows the internal microprocessor to perform its built-in logic and error-checking functions. Manual keypad entry remains undisturbed. Operation of the interface uses simple parallel input/output, so there are no restrictions on the choice of laboratory microcomputer or programming language. Timing restrictions are minimized, since the keypad array circuitry is designed to accept asynchronous, variable-length activation (i.e., manual depression of a key). Other advantages include low cost and the need for only a few chips. By far the best feature, however, is that the emulator operates independently of the rest of the instrument, so only a minimal knowledge of the instrument circuitry is necessary for implementation.

For use in a microprocessor-based instrument, a keypad array must have driver and receiver circuits as shown in Figure 7. The driver circuit scans across the columns of the array, activating each column in turn by sending it a logic-level pulse. If a key is depressed, the pulse is transmitted along the proper row to the receiving circuit,

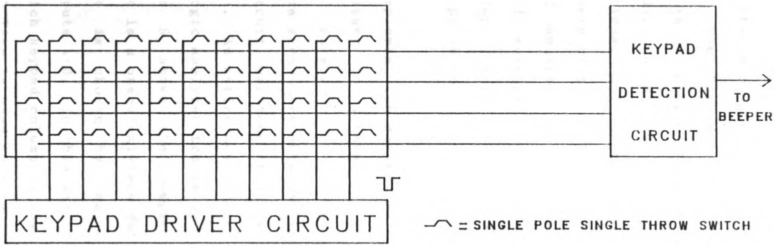
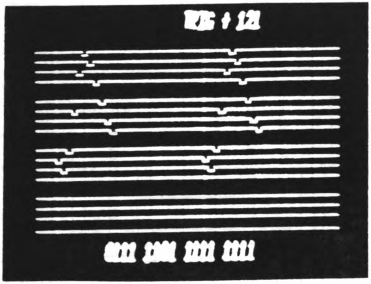


Figure 7. Keypad array for the SP8700 solvent delivery system.

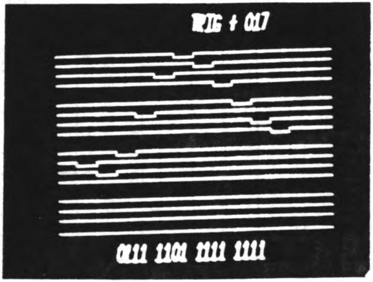
where it is sensed and acted upon. By knowing the column that was activated and the row from which the pulse was received, the microprocessor can deduce which key was depressed. The keypad driver and receiver are often contained in a single IC as in the National Semiconductor MM5740 (115). The activating pulses are active-high in some implementations, but active-low in others. Whatever the specific logic of the driver circuitry, the general strategy for keypad emulation is the same. The column selection is made by an IC multiplexer, and the row selection by an IC demultiplexer. This provides an alternate path for the activating pulse to travel from the driver to the receiver circuit.

To implement the keypad emulator on the SP8700, it was only necessary to determine the logic level of the activating pulse. The characteristics of the signals entering the array were obtained with an oscilloscope. The driver circuit was found to supply active-low pulses 85 μs in duration. An entire scan cycle was completed in 2.420 ms. The logic analyzer traces are shown in Figure 8.

Figure 9 shows the complete interface scheme. The multiplexer is a one-of-sixteen data selector (74150). The input line is chosen by the logic levels established at control inputs A-D. In this way, four PIO lines are used to select which keypad column is the input to the multiplexer. A fifth PIO line is used to gate the interface on and off by



500 μs/div



125 μs/div

Figure 8. **Keypad driver timing diagrams. Pulse length = 0.085 msec. Cycle period = 2.42 msec.**

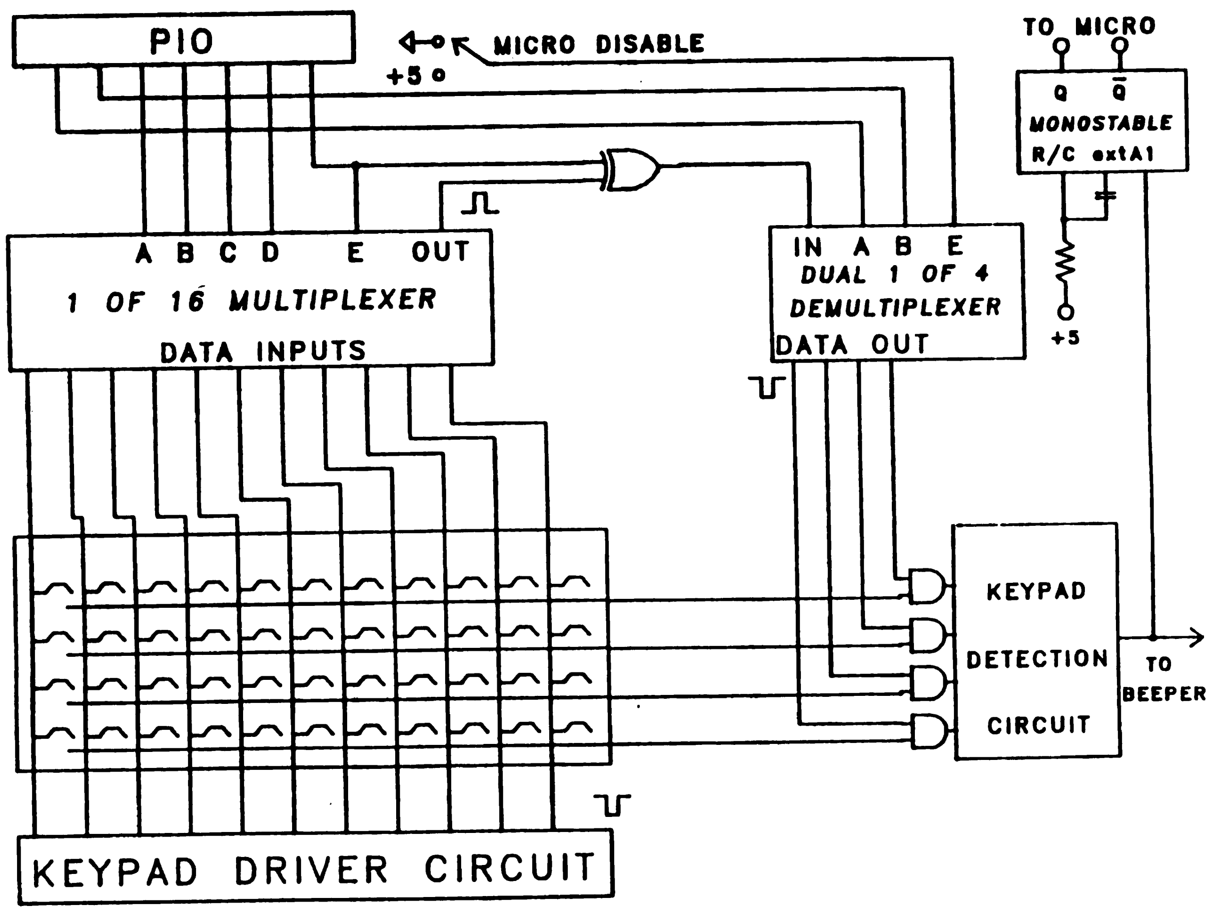


Figure 9. Keypad emulator circuit.

using the multiplexer "chip enable" input (E, active-low). When the multiplexer is enabled, the inverted column pulse is transmitted through a logic gate to the demultiplexer, which is a dual 2-line to 4-line decoder (74155). This chip can be used in several ways, but the implementation shown uses an inverting input to obtain the original pulse logic at the output. The A and B inputs are control lines. These are attached to two PIO lines which select the proper row to which the pulse is transmitted. The E input on the demultiplexer is an active-low enable input, tied to a single-pole, double-throw toggle switch which can be used to disable microcomputer control completely. Thus, only 7 of the 8 PIO lines are needed to control an array of this size.

The AND gates shown on the inputs to the receiving circuit allow for simultaneous manual keypad entry. The exclusive-OR gate shown between the multiplexer and demultiplexer acts as a selective inverter to ensure that all outputs of the demultiplexer are high when the multiplexer is disabled. Thus, low pulses arriving by either path are transmitted through the AND gates to the receiving circuit.

Although this implementation was designed for driver circuits that produce active-low pulses, the strategy for active-high pulses is similar. The scheme in Figure 9 can be modified for active-high pulses by replacing the AND gates with OR gates and putting inverters on the demultiplexer outputs. A non-inverting input is then used on the demultiplexer to restore the original pulse logic after the inverters.

Although not essential to the operation of the interface, a method to verify entries was desired. Many microprocessor-based instruments provide an acknowledgement signal to inform the operator that an entry was valid. The SP8700 uses a beeper which is triggered by a short active-low TTL pulse. A monostable multivibrator (74121) was added to convert the trigger pulse into a 13 ms active-high pulse appropriate for our laboratory microcomputer.

Figure 10 shows a simple flow-chart for operation of the keypad emulator. To simulate one keystroke the microcomputer sends the control code for that key to the PIO port, with the control line to the multiplexer held low. For example, if the multiplexer code for the column is 0110 and the demultiplexer code for the row is 01, the binary word 0101100, or decimal 44, would be sent to the PIO port. A complete list of key codes is shown in Figure 11.

The key code is maintained at the PIO port for at least one complete array-scan cycle. If an acknowledgement is not being utilized, the emulator is disabled by sending a high logic level to the least significant PIO line. The status of the other lines of the PIO is unimportant during the disable period. The microcomputer is then free to send the next control word, or to return to the calling routine.

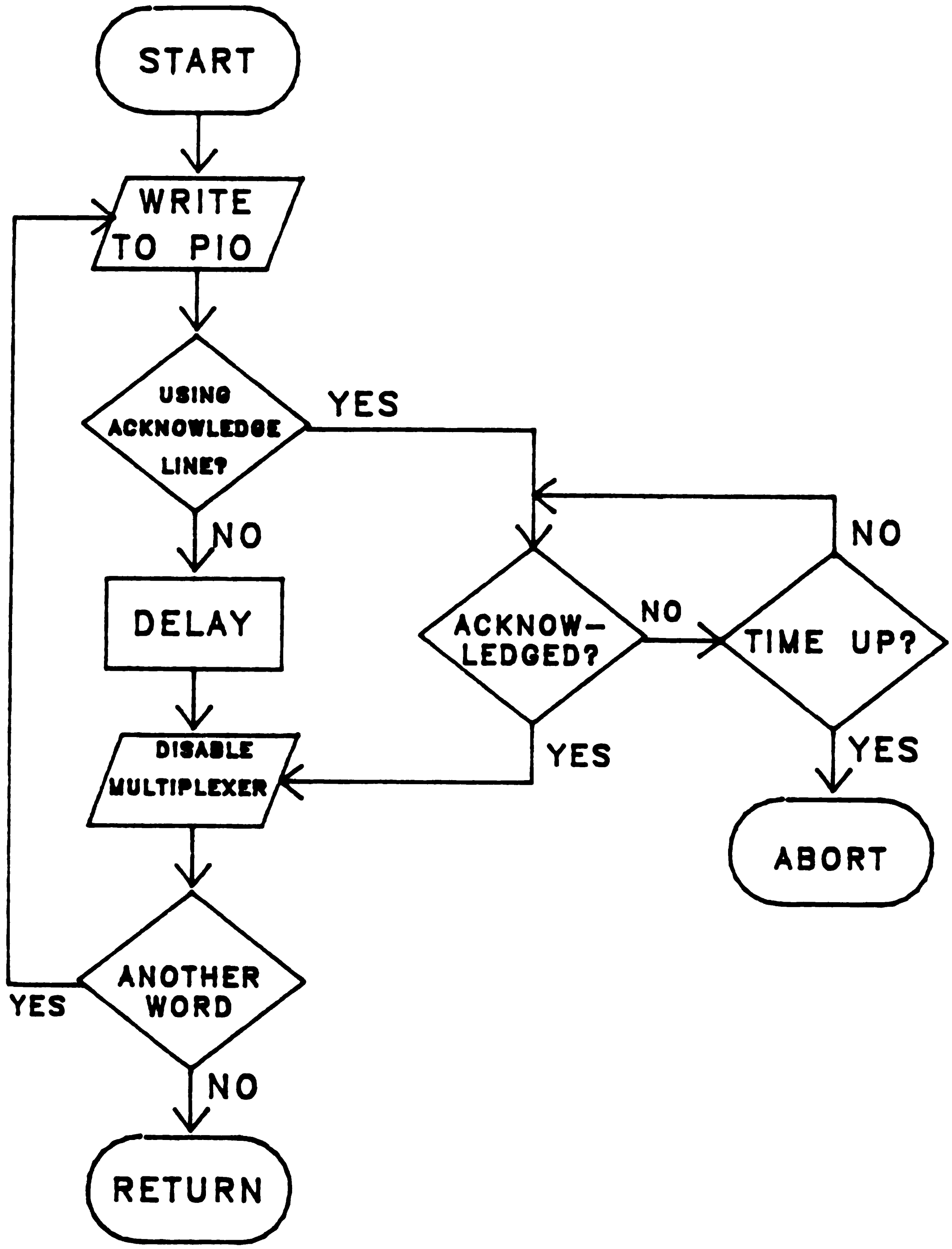


Figure 10. Flowchart of keypad emulator operation.

74150 CODE DCBA / 74155 CODE BA	0110	0111	1001	1010	1000	0101	0010	0000	0001	0011	0100
00	EDIT RUN		TIME	%A	%B	%C	FLOW	MAX PRESS	7	8	9
11			CHANGE TIME	EDIT FILE	STORE FILE	DELETE LINE	↓	CLEAR ENTRY	4	5	6
10			INIT FILE	RUN GRAD	HOLD	CONT	PURGE	STOP	1	2	3
01	TEST			DISPLAY	PRESS				0	.	ENTER

Figure 11. Key codes for the SP8700 keypad emulator interface.

The implementation shown for the SP8700 makes use of an acknowledge signal to determine if a keystroke was valid. In this case, if the keystroke is not acknowledged within one scan period, the laboratory microcomputer can perform some other action. This lack of acknowledgement usually happens when invalid parameters are entered and discovered by the logic and error-checking routines of the instrument. The emulator itself has been 100% successful in simulating the proper keystroke.

4. Valving Requirements for MHPLC

Valves used for column selection must satisfy two important requirements for use in multidimensional HPLC. First, the valve and associated fittings must withstand the pressures produced by all columns used in the system. The maximum pressure expected under normal circumstances is 5000-6000 psi. Beyond this range most HPLC pumps will not operate properly, so valves with higher pressure limits would be of limited utility.

The second major requirement is that the valve should be of low dead-volume design. This is normally not a problem with standard-bore (4.6 mm) columns, but becomes of greater concern as one uses smaller inside diameters. Of equal, or even greater concern, is the tubing used in connecting the valves to the columns. Since some lengths of tubing used must be at least as long as the column, it is important to use narrow-bore (0.009") tubing for connections beyond the injector valve. Valves which have been designated as suitable for injection applications should also be suitable for column-switching purposes. In this system, we have found the Rheodyne 7000 series valves to be well suited for column-switching. These valves operate at pressures up to 7000 psi (480 bar) and contain 0.024" diameter flow passages.

A third feature that is highly desirable in a column-switching valve is remote actuation and sensing. Two types of actuators are commercially available: motor driven and pneumatic. Of the two types, the pneumatic variety is more common, mostly due to lower cost and faster switching times. To operate the pneumatic actuator, a 2-position, 2-port solenoid valve is used to control the air flow to either side of the actuator piston. Air or nitrogen at 50-70 psi is used to provide the driving force to switch the HPLC valve. Figure 12 shows a schematic of the valve control circuitry used to switch valves under microcomputer control. The solenoid valve operates at 120 volts AC and is rated for continuous duty operation. Several manufacturers can supply the solenoid valves; the particular valves installed on this system were obtained from Scovill Corporation (model 1-1115, Wake Forest, NC). An optically isolated relay (Teledyne 611-3, Hawthorne, CA) is used to operate the solenoid valve. A three position toggle switch

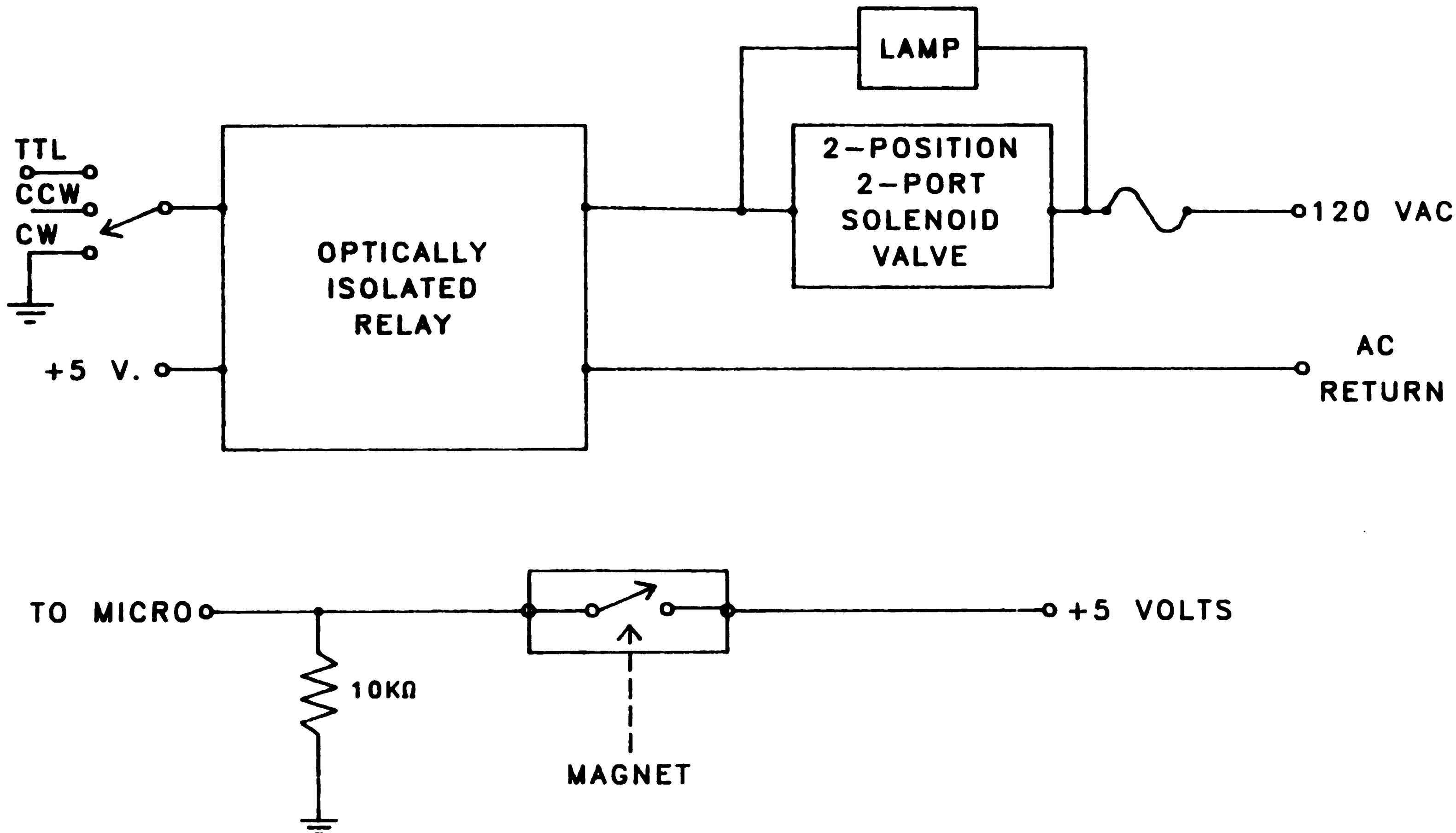


Figure 12. Valve control and sensing circuits.

is connected to the input of the relay; two positions are used for manual control of the valve position and the position labeled "TTL" is connected to the PIO port of the microcomputer. A low level at the PIO line turns the solenoid valve on.

The position of the valve is determined by the state of a magnetically operated reed switch mounted on the valve housing. A magnet is mounted on the valve shaft and moves into proximity of the reed switch when the valve is in the clockwise position, thus closing the switch. A change of state thus occurs at the input bit of the PIO, enabling the microcomputer to determine if a valve rotation was successful.

Several different styles of valves have been used for multidimensional HPLC, but we have found a combination of two 6-port, 2-position valves to be the most versatile. Figure 13 shows several ways a single valve of this type can be connected for a variety of applications. Two valves of this type allow the user to configure the system over 30 ways, which should accomodate a considerable number of switching applications. The autoinject and fraction collection configurations, although not strictly column-switching in nature, are nonetheless valuable applications of the system. The autoinject mode allows the user to repeatedly inject a sample contained in a sealed sample vessel. The sample is forced into the injection loop by the same air pressure that drives the actuator piston.

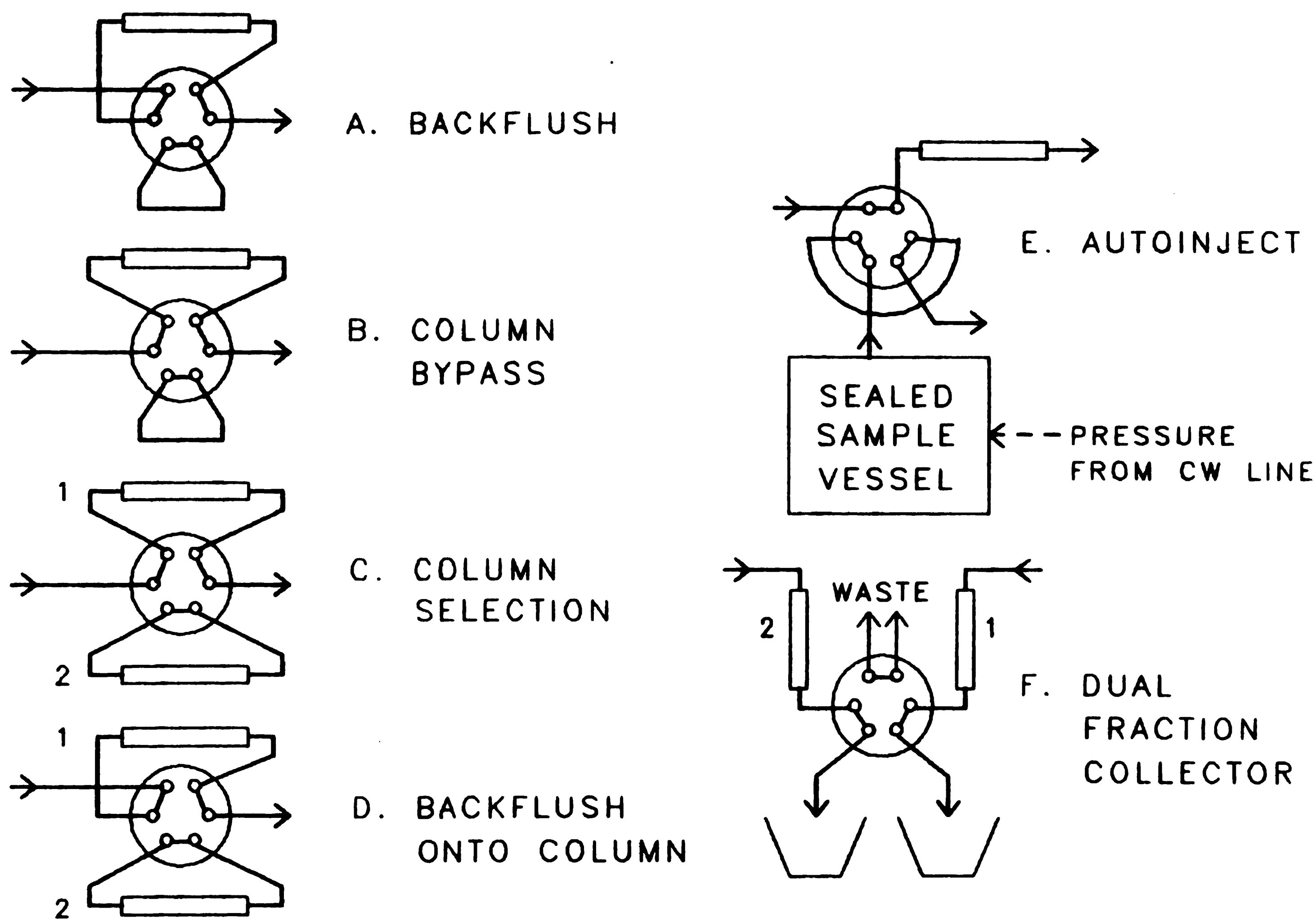


Figure 13. Configurations of a 6-port, 2-position valve.

This mode is useful for automated methods development or fraction collection.

5. Column Requirements

The columns used in multidimensional HPLC are subjected to higher pressures, sudden changes in pressure and reversals of flow direction. For these reasons, packing stability is an important consideration when choosing columns for switching applications. Packings which are based on spherical particles are much less prone to developing voids and channels, so this type of packing is preferred. Columns should have frits (preferably removable) at both the inlet and the outlet of the column. If these requirements are satisfied, the column should be able to be reversed without significant loss of separating ability. Columns of this type which have been used successfully include a Perkin-Elmer High Speed 5 micron silica column, and Alltech Spherisorb 5 micron particle size columns (available in various lengths and bonded phases).

Columns which are packed with gel-type materials can be used in a multidimensional system, but flow reversal is not recommended. Care must also be taken to minimize pressure shocks to these columns when another column is switched into or out of line past the gel column. This can be accomplished by installing a flow restrictor on the alternate flow path. When the column is switched, the backpressure seen by the fragile column will remain fairly constant. Examples of this approach can be seen in the applications section of this dissertation.

B. Isolated Droplet Generation

1. Theory and System Overview

As mentioned previously in the introduction and historical sections of this dissertation, an interface which could deliver HPLC effluent to a flame or plasma with little dead volume and high efficiency would be an invaluable addition to element specific detection. To this end, an isolated droplet generator (IDG) was constructed based on the principles discovered by Rayleigh in 1879 (85-86) and further advanced by Schneider et al. (95-96) and Hieftje et al. (99-105). The device described below is a significant improvement over previous designs and is based upon the induced breakup of a liquid jet.

Figure 14 shows the basic components of the IDG. A liquid is forced through a glass capillary with a velocity sufficient to cause the formation of a liquid jet. The capillary is attached to a rectangular piezoelectric crystal called a bimorph. When an oscillating voltage is applied to the bimorph, it causes the crystal to vibrate at the oscillation frequency. These vibrations are transmitted through the capillary to the surface of the liquid jet.

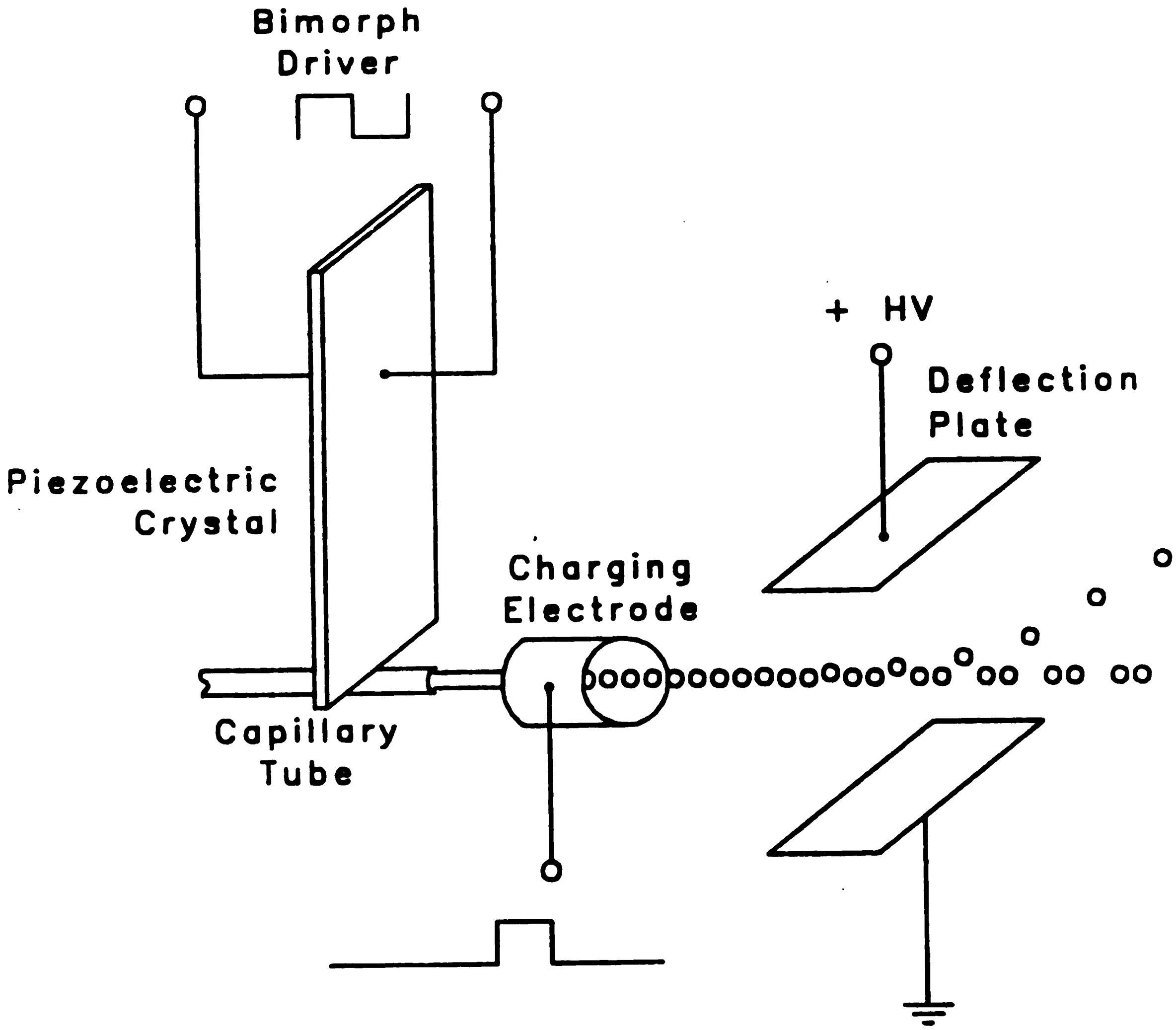


Figure 14. Basic components of the isolated droplet generator.

Normally, the jet would travel in a cylindrical mass for some distance before it disrupts into a series of small drops of random size and spacing. The disruption occurs because the liquid jet, as it travels from the capillary tip, becomes dynamically unstable under the action of surface tension. When a periodic disturbance is launched onto the surface of the jet, disruption again occurs. If the wavelength of the disturbance is greater than the circumference of the jet, however, the disintegration occurs in a uniform manner, and produces droplets of uniform size and spacing.

In most instances the optimum droplet production rate is faster than that desired experimentally. In this case the rate of droplet delivery can be slowed by trapping unwanted droplets by selective charging and deflection. A cylindrical charging electrode is placed at the point where droplets are forming from the liquid jet. If a voltage is applied to the electrode when the droplet breaks from the jet, a charge is imposed on the droplet by a process of induction. High voltage deflection plates are then used to deflect the charged droplets out of the main stream.

The instrumentation used for droplet generation has been entirely constructed in-house with the exception of the power supply for the high voltage deflection plates and the strobe lamp used for viewing the droplets. The high voltage power supply is adjustable from 0 to 10,000 V at 3 mA (Model

205A-10P, Bertran Associates, Inc., Syosset, NY). A voltage of 4000 V is usualy sufficient for droplet deflection. The strobe lamp is capable of 25,000 flashes per minute and can be externally triggered (Model GR1531AB, GenRad, Inc., Concord, Mass.).

Several good discussions of the theory of droplet production are available (88,95,97-98,116). The pertinent equations are summarized below; the reader is referred elsewhere for the complete derivation (98).

In considering the various energy contributions involved in the production of uniform-sized droplets Rayleigh included the potential energy due to surface tension, the increase in energy at specific locations on the jet caused by jet distortion from the launched disturbance, the static potential energy caused by the electrical charge on the droplets, and the kinetic energy of motion of the liquid. Rayleigh then formulated that the cylindrical surface of the liquid jet could be represented by the equation:

$$r = A_0 + C \cos(2\pi z/\lambda)$$

where r is the liquid jet radius, A_0 is a variable confined by jet dimensions, C is a small quantity variable with time, and λ is the wavelength of disturbance.

The jet is predicted to have a maximum and minimum radius of $A_0 + C$ and A_0 respectively, with spheroid-shaped masses separating each minimum in radius. By plotting certain factors describing jet instability as a function of the jet circumference, Rayleigh showed that the most rapid instability occurs when the wavelength of the launched disturbance equals 9.016a, where a is the liquid jet radius. The jet radius can be calculated from the capillary inside diameter if the coefficient of contraction is known. Lindblad and Schneider (97) have measured the coefficient of contraction for aqueous solutions and found a value of 0.86. Therefore the wavelength of most rapid instability can be calculated as:

$$\lambda_m = (9.016)(.86)(.5)\ D = 3.88\ D$$

where D is the capillary diameter (cm).

This wavelength should produce drops at the shortest distance from the capillary tip. The frequency of oscillation f_m corresponding to this wavelength can be found by dividing the the velocity of the liquid jet v_J (cm s^{-1}) by the wavelength:

$$f_m = v_J / \lambda_m$$

The droplet size produced can be varied over a limited range by varying the applied wavelength. The theoretical lower limit is constrained by the fact that the applied wavelength must be at least as large as the circumference of the jet for uniform breakup to occur. Although there is no theoretical upper limit of wavelength, in practice one begins to see the effects of harmonics when the wavelength exceeds about 14*a*. If the fundamental drive frequency is too low, droplets of several sizes can be produced from the same capillary. This phenomenon is exploited in the present design of the IDG, which uses a square wave to drive the transducer as opposed to a sinusoidal waveform. Because of the distribution of harmonics in the square wave, uniform droplets can be made at much lower frequencies.

The droplet volume V_d (mL) can be calculated by measuring the bulk flow rate F (mL s^{-1}) through the capillary and dividing by the production frequency, f (s^{-1}):

$$V_d = F/f$$

Since the volume of a sphere is given by the equation:

$$V = (4/3)\pi r^3$$

the drop radius can be expressed as:

$$r_d = [(3\ F)/(4\pi f)]^{1/3}$$

The overall error in the determination of the drop radius by this method is reported to be of the order of 0.33% (97).

In summary, the parameters affecting droplet production are capillary size, bulk flow rate through the capillary, and applied transducer frequency. The capillary size is adjusted to produce droplets in a given size range, and the transducer frequency is used to vary the size within this range. The bulk flow rate can also be adjusted, but droplet charging is more efficient at low linear velocities.

2. Capillary production and mounting

A critical step in the construction and operation of the IDG is the production of capillaries suitable for forming the liquid jet. Two options are possible: purchase of a continuous-bore capillary of the appropriate inside diameter, or constriction of a larger bore to narrow the exit. For larger capillaries (100 μm or greater) a continuous-bore variety should work nicely. A good source of this size capillary is vitreous silica capillary tubing of the type used for capillary gas chromatography work (Scientific Glass Engineering, distributed by Anspec Co., Ann Arbor MI). For smaller capillaries, the continuous bore creates excessive backpressure. This can be avoided by using a larger bore with a constricted exit.

Larger bore capillary material to be constricted should be made of a soft glass capable of being fire-polished. Disposable micro pipets (Dade Hospital Supply Corp., Miami, Fla., 5 μL size) were found to be suitable sources of controlled bore tubing for this application. The end of the pipet is fire-polished in a cool flame and periodically checked with a microscope or reticle until the desired diameter is obtained. Care must be taken to evenly heat the capillary tip, or else a skewed opening will be obtained as shown in Figure 15. The geometry of a properly fire polished tip is shown in Figure 16. Capillaries 100 to 50 μm in diameter are easily made by this method. With

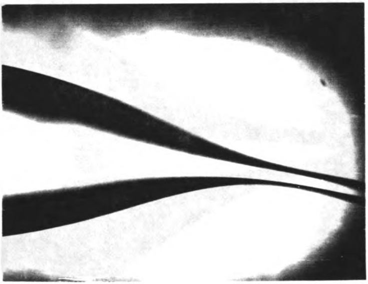


Figure 15. An improperly fire-polished capillary tip (43 micron diameter).

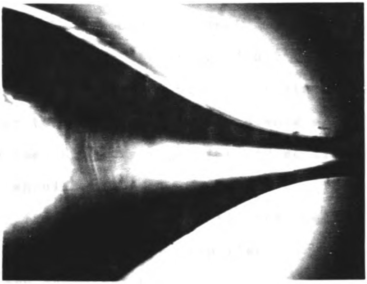


Figure 16. A properly fire-polished capillary tip (83 micron diameter).

practice, diameters as small as 10 μm can be obtained, but excessive backpressure prevents these from being experimentally useful. A more realistic lower diameter limit for useful capillaries is 25 μm. Acid etching of the capillary tips may be helpful at very small diameters. Before use, the capillaries must be flushed in the reverse direction to wash out any particles which may clog the constriction. To facilitate wash-out of the tubing, capillary lengths should be kept as short as possible. Typical lengths were on the order of 0.5 to 1.0".

The capillaries were mounted by gluing the capillary perpendicular to the bimorph at a distance of approximately 0.25" from the capillary tip. A rigid-setting glue such as Duco Cement should be used to facilitate transfer of the vibrations from the bimorph to the capillary without dampening. After gluing, the capillary-bimorph assembly is allowed to dry overnight before using.

3. Constant Pressure Liquid Delivery Vessel

Figure 17 shows a diagram of the liquid delivery system used to form the jet when the system was not used as an HPLC interface. The system consisted of an aluminum cylinder with a threaded brass cap containing a viton rubber seal. For delivery of solutions incompatible with aluminum, a polyethylene insert was placed within the aluminum cylinder. Regulated gas pressure at 10 to 60 psi was provided through

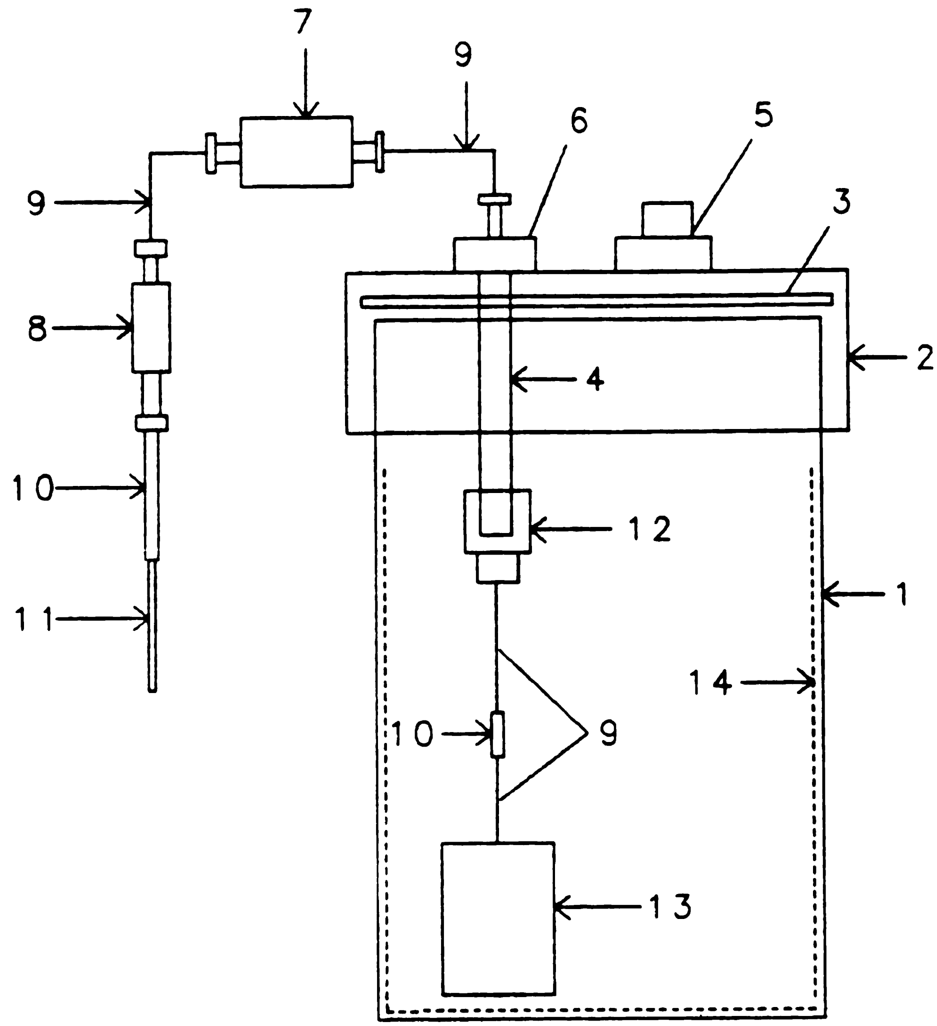


Figure 17. Solution delivery reservoir. 1: Aluminum cylinder, 5" x 3" o.d. x 2.6" i.d. 2: Brass cap 0.75" x 3.25" o.d. 3: Viton rubber seal. 4: Copper tubing 1/4". 5: 1/4" Swagelok fitting. 6: 1/16" inverted fitting. 7: 2 μm in-line filter. 8: 1/16" SS to 1/16" cheminert adapter. 9: 1/16" SS tubing. 10: Microline tubing. 11: Glass capillary. 12: 1/4" to 1/16" Swagelok union. 13: Inlet filter. 14: Polyethylene liner (optional).

a fitting in the cap, forcing liquid through a sintered metal filter to a 1/16" fitting also in the cap. Attached to the outlet fitting is a 0.45 μm in-line filter of the type used in HPLC (Rheodyne 7335). After the filter is a union which adapts from 1/16" stainless steel tubing to 1/16" plastic tubing compatible with Cheminert fittings. The plastic tubing was 0.04" i.d. and 0.07" o.d., which is a size easily force-fit onto the end of the capillary. A number of tubing materials were evaluated for solvent compatability, and Microline tubing (Thermoplastic Scientifics, Inc., Warren, NJ) was found to be compatible with all HPLC solvents, yet still flexible enough to be force-fit.

4. Bimorph and Electrode Mounting Assembly

Figure 18 shows the method used to hold the electrodes and bimorph. The mounting plate was constructed of 1/4" Plexiglas. The high voltage electrodes were constructed of brass dipped in a plastic insulation (Plasti-Dip, PDI, Inc., St. Paul, MN) for safety. Brass bolts were soldered to the electrodes and used both for mounting to the Plexiglas and for electrical connection. The circular charging electrode was also constructed of brass and was mounted with a Teflon arm to a brass bracket attached to the rear of the Plexiglas plate. Electrical connection to the charging electrode was made by directly soldering a wire to the electrode. The

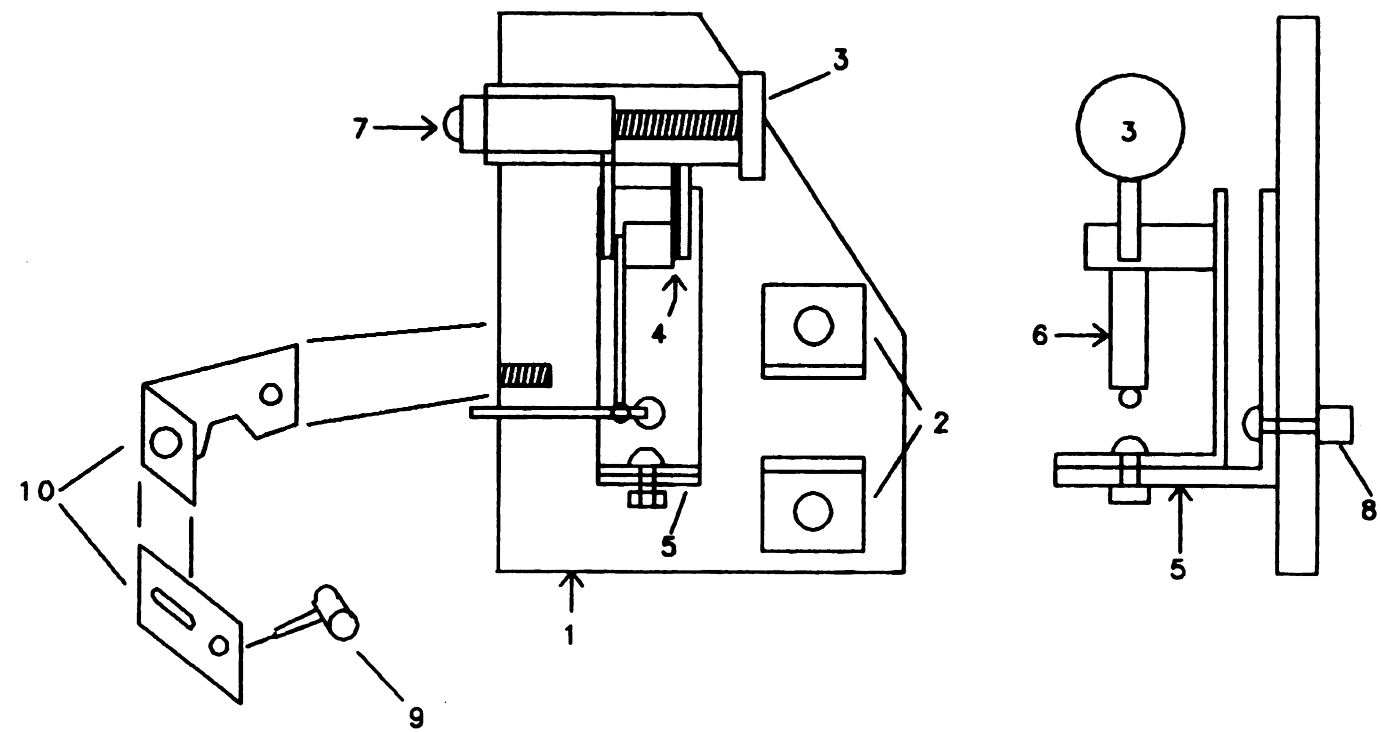


Figure 18. Bimorph and electrode mounting assembly (shown approximately 1.2 times enlarged). 1: 1/4" Plexiglas. 2: High voltage deflection plates. 3: Screw clamp. 4: Insulation. 5: Gimbal mount for positioning capillary. 6: Bimorph. 7: Electrical connection for bimorph. 8: Bimorph ground connection. 9: Cylindrical charging electrode 1/4" x 1/4" o.d. x 1/8" i.d. 10: Charging electrode positioning bracket.

bracket holding the charging electrode can be adjusted to give vertical, horizontal and side-to-side movement. The electrode itself can also be rotated by turning the Teflon connecting arm.

The bimorph mount was constructed to provide rotation in the horizontal and vertical planes while maintaining the tip of the capillary stationary. This rotation ability can correct for errors in mounting the capillary or errors caused by uneven heating during fire-polishing of the capillary tip. Thus, even if the jet is not in perfect alignment with the capillary axis, it may still be usable. Electrical connection to the bimorph is made by clamping it between the rotating mount and the fastening bracket. The mount is held at ground potential, while the fastening bracket is connected to the bimorph driver. The present design was adapted from a mount proposed by Seymour and Boss (117), but is considerably smaller and has more movement of the charging electrode.

5. IDG Internal Electronic Circuitry

Figure 19 shows a block diagram of the system as it is configured for stand-alone production and charging of droplets. A more versatile computer-controlled configuration has also been implemented and is discussed in detail below.

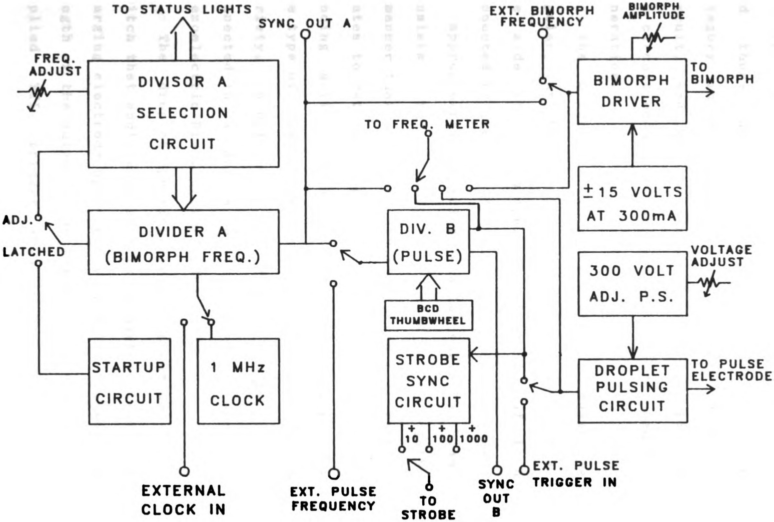


Figure 19. Block diagram of the stand-alone version of the isolated droplet generator.

The stand-alone version of the IDG can be divided into two types of circuits: those used for waveform generation and those used for waveform translation. The latter category includes the bimorph driver, droplet pulsing circuit, and strobe synchronization circuit. The rest of the circuit modules shown in Figure 19 are used for waveform generation.

The bimorph driver converts a TTL square wave into a bipolar, variable amplitude square wave which is applied to one side of the bimorph. The other side of the bimorph is connected to ground. The amplitude is adjustable from zero to approximately 26 volts peak-to-peak. The bimorph itself consists of two piezoelectric plates bonded together in such a manner that a voltage applied to the electrodes causes the plates to deform in opposite directions. This results in a bending action that is a function of the applied potential. One type of bimorph which performs well is the PZT-5H variety, 0.021" X 0.12" X 1.75" with the electrodes connected in a series positive configuration (Vernitron Piezoelectric Div., Bedford, OH).

The droplet pulsing circuit is basically a transistor switch that applies a fast-rising high voltage pulse to the charging electrode upon receiving a TTL trigger signal. The length of the pulse is determined by the length of the applied TTL trigger input. The charging voltage is adjustable from zero to approximately 300 V.

The strobe synchronization circuit acts as an interface between the IDG and the strobe lamp used for illumination of the droplet stream. The strobe flash is synchronized to droplet production by another TTL trigger signal which closes a transistor switch, shorting the external trigger output of the strobe to ground.

The other circuits function to produce the waveforms necessary for driving the translation modules described above. Two requirements are necessary for reliable droplet generation. First, the waveforms must be very stable in frequency. Any drift or discontinuities will drastically alter the nature of the droplet stream. Second, the waveform used for droplet charging must be synchronized with that used for droplet production or the amount of charge delivered to each droplet will vary, causing the pulsed droplets to be deflected unevenly. To meet these two requirements, a crystal oscillator was used as the primary frequency generator. This 1 MHz TTL square wave is digitally divided to produce a lower frequency square wave which is sent to the bimorph driver. The frequency of the square wave used to drive the bimorph will be the fundamental frequency of droplet production.

The bimorph driver input signal is also sent to a second divider which is used to select the frequency of droplet charging. The number entered on the BCD thumbwheel corresponds to the number of drops allowed to pass through uncharged before the pulsing electrode is turned on for one droplet production cycle.

Each of the above circuit modules is discussed in detail below.

Bimorph Driver Circuit

Figure 20 shows a schematic diagram of the circuit used for driving the piezoelectric element. The trigger waveform is connected at the point labeled "TTL IN." This is capacitively coupled to one input of an LM311 comparator which is used to amplify the TTL input and make it bipolar. The 1 MΩ resistor connected to -15 V prevents the comparator from oscillating if the input is disconnected. The output of the comparator is adjustable from approximately $\pm$ 7 to $\pm$ 15 V by varying the 10 kΩ potentiometer connected to pin one. The output of the comparator is capacitively coupled to a two-stage, complementary symmetry transistor amplifier made up of transistors 2N3904 and 2N3906 as the first pair and 2N5192 and 2N5195 as the second pair. This amplifier does not alter the voltage imposed upon the bimorph significantly. Its main function is to provide the higher currents necessary to efficiently drive the low impedance bimorph and overcome the natural resonances of the piezoelectric element.

The $\pm$ 15 V power supplies connected to the bimorph driver are dedicated to this circuit alone to ensure that

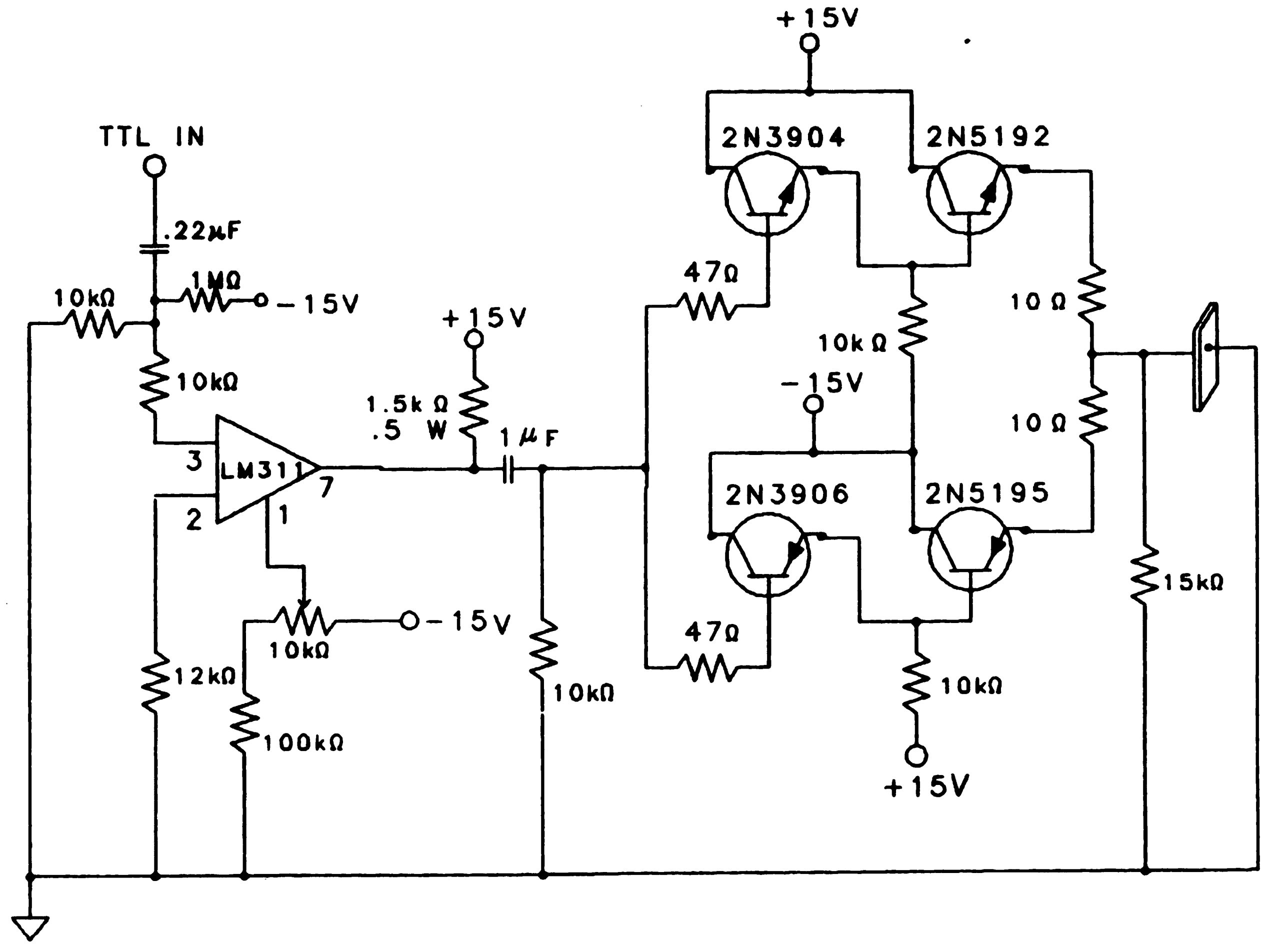


Figure 20. Bimorph driver circuit.

sufficient current is available to meet the requirements of the piezoelectric element. An approximate current drain can be calculated from the bimorph capacitance. The capacitance of the bimorph was measured in two ways. The passive RC circuit shown in Figure 21 was driven at 10 kHz by a Wavetek function generator with the bimorph in place of the capacitor. The resulting bimorph voltage waveform was noted, and various values of known capacitance were then substituted until an approximate waveform match was obtained. The estimated capacitance by this method was found to be 1500 to 2500 pF. The second method involved connecting the 74121 monostable multivibrator as shown in Figure 21 and observing the pulse width obtained when the bimorph is connected in the place of C_{ext}. The product literature for the 74121 estimates the pulse width from the equation:

$$T_{pulse} = C_{ext} R_{ext} \ln 2$$

The measured pulse width of 0.0148 ms was substituted into this expression to solve for the capacitance of the bimorph. The value obtained by this method was 2170 pF. Using this value one can estimate the current required by the relation:

$$i = C\, dV/dt$$

where i is the current and dV/dt is the fastest voltage change, which will occur at the edge of the square wave.

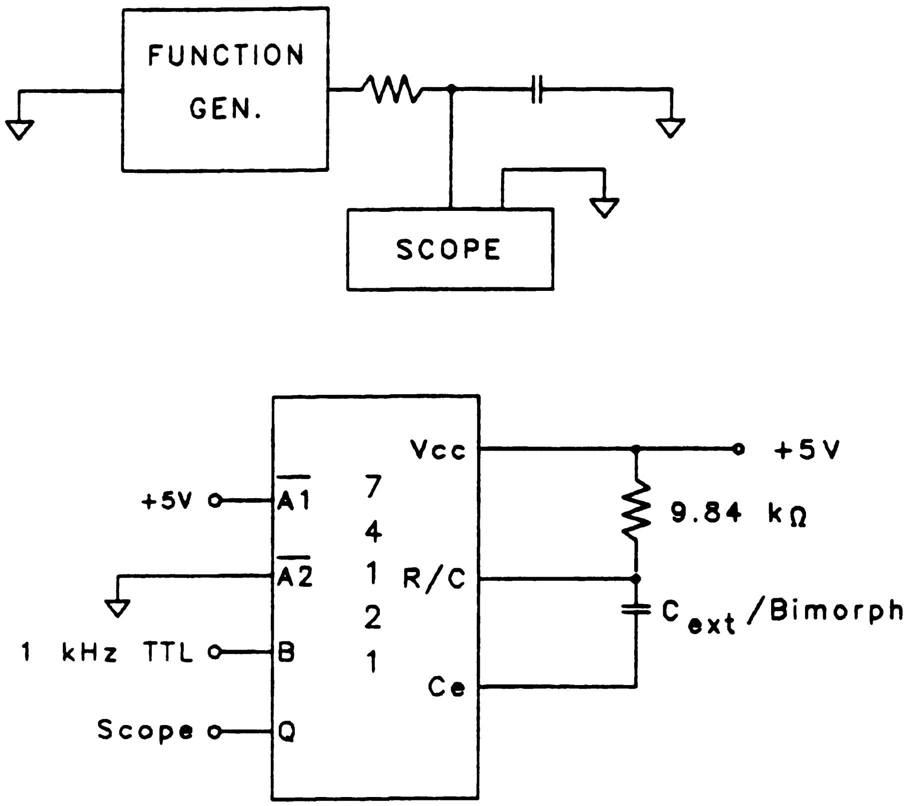


Figure 21. Circuits used to measure bimorph capacitance.

Estimating a 30 V change to occur in approximately 0.001 ms, the maximum current required would be 65 mA. Since this is a rough estimate, a 300 mA power supply was installed; this should provide a good margin for error in the estimate.

Although the power supply should be capable of supplying current for operation up to 100 or 150 kHz, in practice the heat sinks installed on the transistors are only capable of dissipating the heat generated from a maximum operating frequency of about 80 kHz. For most experimental applications, however, droplet production frequencies of less than 50 kHz are sufficient. Figure 22 shows the shape of the waveform produced with the bimorph installed. Figure 23 shows the relationship of the peak-to-peak and flat-to-flat voltages for various settings of the front panel bimorph driver amplitude adjustment.

Droplet Pulsing Circuit

Figure 24 contains a schematic diagram of the circuit which is used to control the voltage present at the electrode used for droplet charging. A TTL input is applied to the base of control transistor SK3044. When the input is in the high state (+5 V) the control transistor turns on the pass transistor SK3053, allowing current to pass through the 30 kΩ resistor to ground. In this manner, the voltage present at the collector of the pass transistor is presented to the charging electrode. When the TTL input is low (0 V),

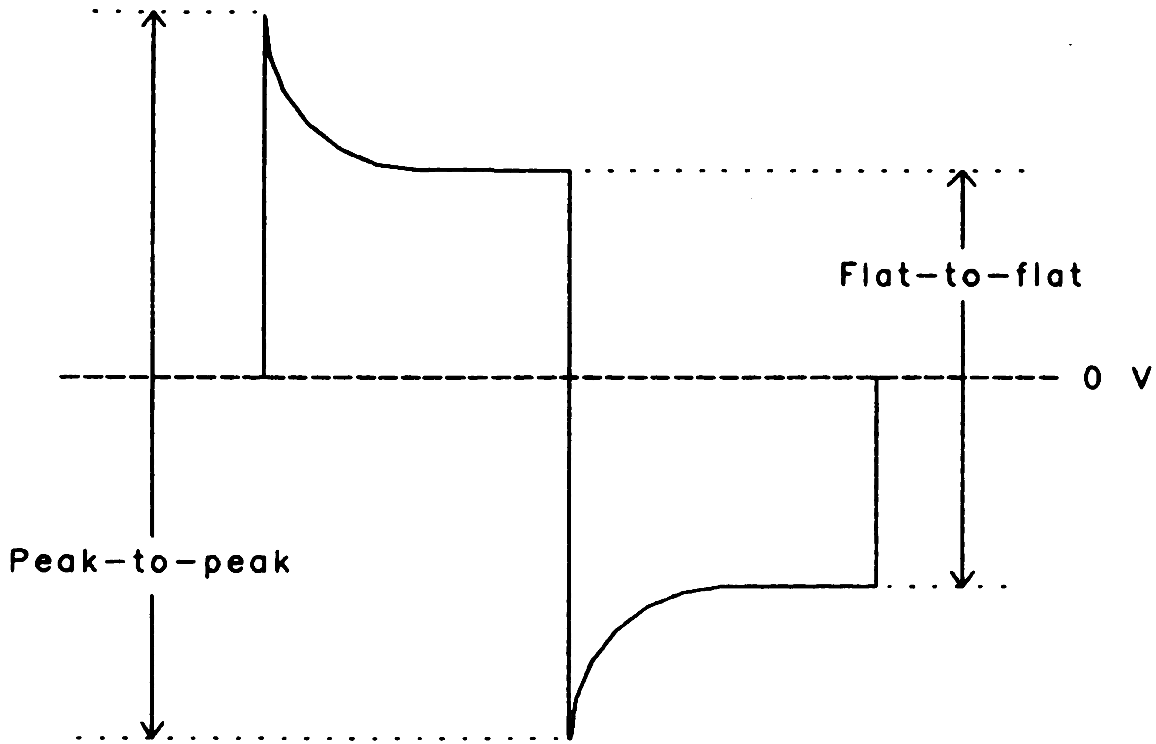


Figure 22. Bimorph driver output waveform.

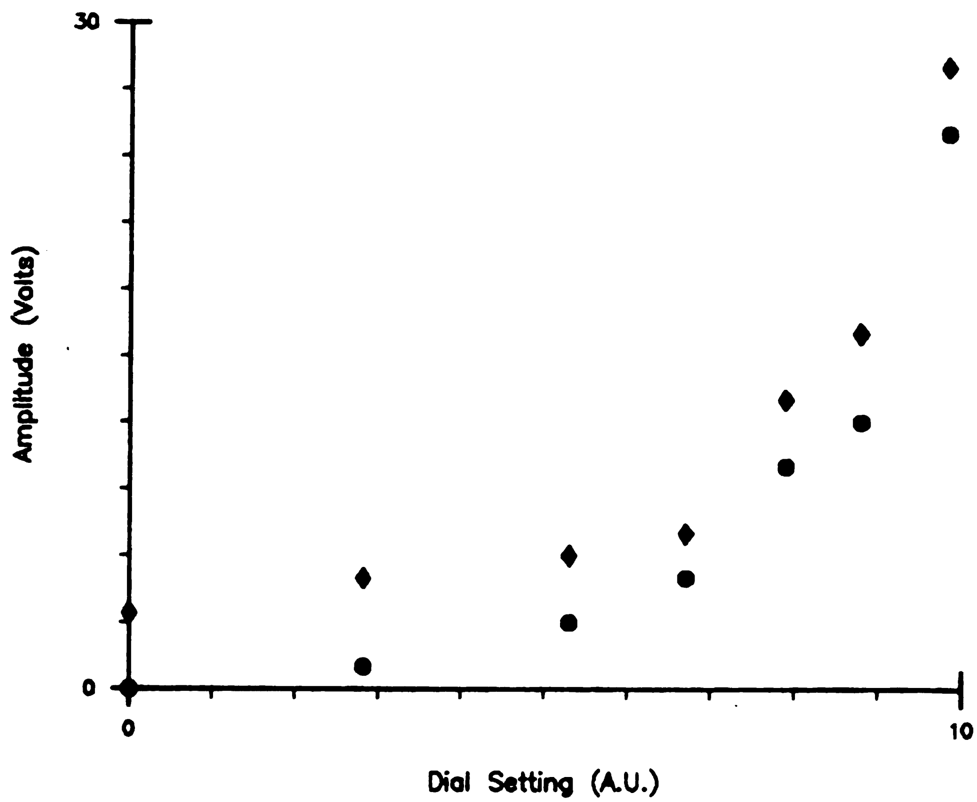


Figure 23. Bimorph driver amplitude for various dial settings. (♦) Peak-to-peak voltage. (●) Flat-to-flat voltage.

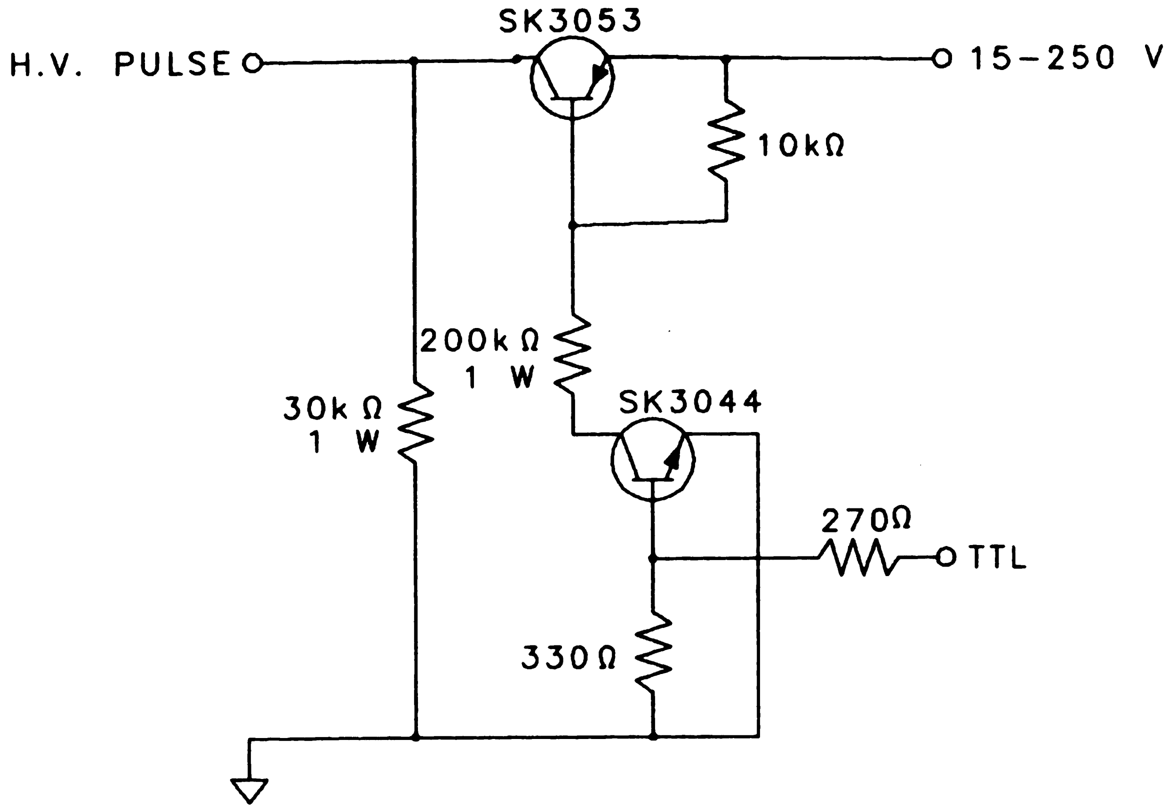


Figure 24. Droplet charging circuit.

both transistors are off, and the charging electrode is held at ground potential through the 30 kΩ resistor.

This circuit provides a good compromise between low current consumption and fast rise and fall times of the high voltage pulse. The relationship between the trigger waveform and the charging waveform is shown in Figure 25. The current consumption for various frequencies is shown in Table 5.

Table 5. Pulsing circuit current consumption.

Frequency (Hz)	Current consumption (mA)
10	3.83
100	3.90
1000	4.00
4000	4.35
10000	5.06
14000	5.60
constantly on	7.62
constantly off	0.00

Strobe Synchronization Circuit

The Strobotac strobe lamp contains an input for firing the lamp externally. The recommended firing method is to use either a 6 V peak-to-peak signal or to simply short the input to ground. The input has approximately 32 V DC present due to the internal voltage divider network of the firing circuit. If a 6 V peak-to-peak trigger is used, the RPM knob must be manually adjusted at each setting to provide adequate bias for firing. The shorting method is

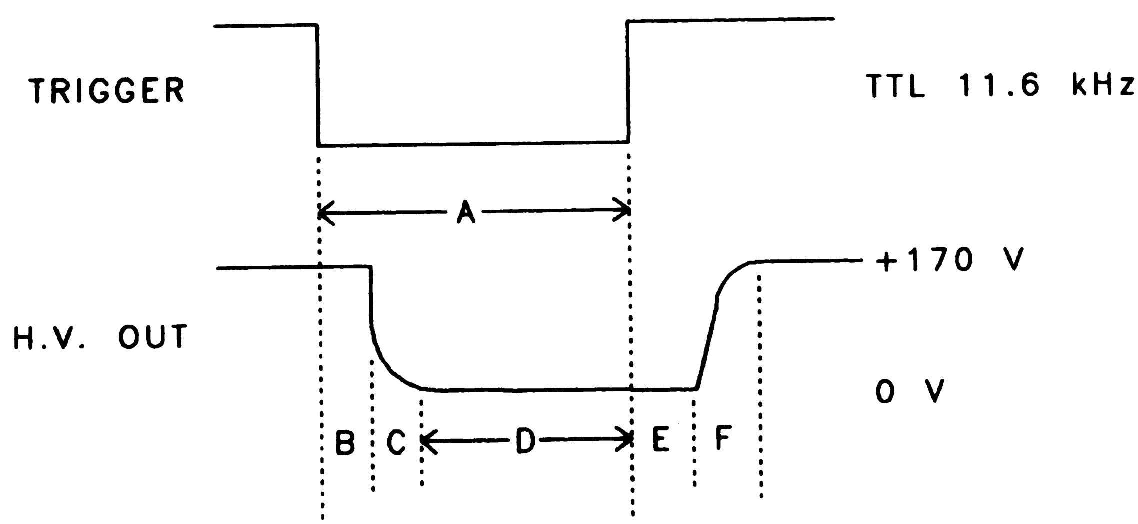


Figure 25. Droplet charging circuit output. A: 43 μs. B: 13 μs. C: 8 μs. D: 20 μs. E: 2 μs. F: 12.5 μs.

therefore superior, since adequate voltage is always present at the trigger input to induce firing, regardless of the range setting. The circuit shown in Figure 26 was constructed to fire the strobe by shorting the input to ground.

Transistor 2N3053A acts as a solid state switch which is closed by an active-high TTL pulse. Because the trigger frequencies are often considerably higher than the maximum firing frequency of the Strobotac (500 Hz), provision has been included to divide the trigger frequency by factors of 10, 100, or 1000. Three 7490 decade counters are cascaded as shown, and a front-panel switch can select between the various dividers. The input to the decade counters is connected to the droplet pulsing circuit input.

Bimorph Frequency Selection Circuit

One disadvantage of previous designs that used digitally synthesized frequency generation was that the frequency was not continuously adjustable. When initially setting droplet production parameters a method of continuously adjusting the frequency is desirable, since the operator must be constantly observing the droplet stream for stability. The circuits shown in Figures 27 and 28 were constructed to provide this feature while also allowing the user to lock in the correct frequency once it was determined. When locked, the output is as stable as the

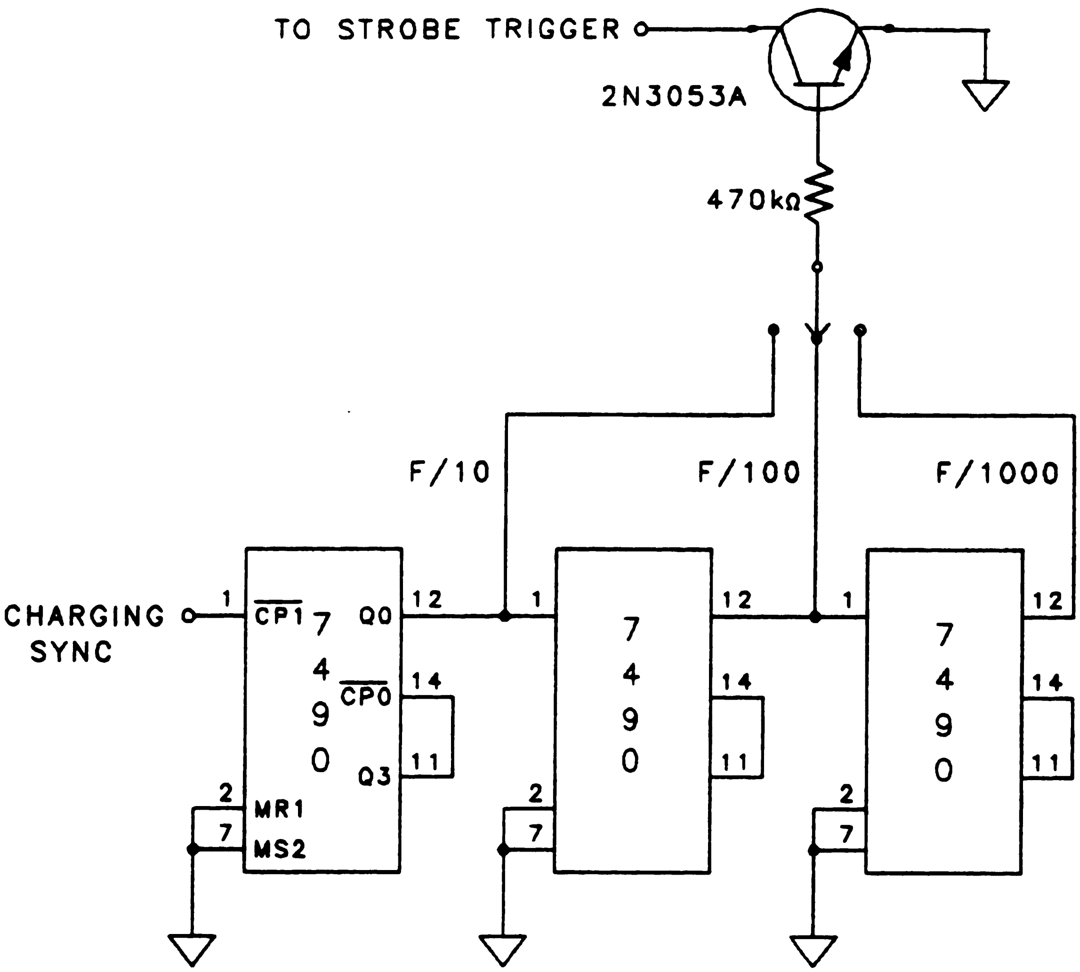


Figure 26. Strobe lamp synchronization circuit.

crystal oscillator input. Because of the complexity of this circuit, the two parts are presented separately. Figure 27 contains those components primarily concerned with divisor selection, while Figure 28 contains the counters used for frequency division.

Selection of a 12-bit frequency divisor is performed with an AD574 ADC. A voltage divider composed of a fixed resistor R, a 5 kΩ trimpot and a 1 kΩ front-panel potentiometer is used to vary the input voltage to the ADC. The value of the fixed resistor is adjusted to set the maximum upper frequency allowable by biasing the lower end of the divider away from ground. The 5 kΩ trimpot is adjusted to set the lower frequency limit and thus determine the total range.

The 1 MHz crystal oscillator provides both the fundamental frequency to be input to the 74191 counters and a trigger waveform used to initiate conversions on the ADC and subsequently load the 74174 latches. A complete conversion/frequency selection cycle involves conversion of the input voltage, latching of the digital output, and loading of the output by the counters. The 1 MHz oscillator frequency is divided by a factor of ten by the 7490 decade counter. This signal is used to trigger a 74121 monostable multivibrator which provides a 38 μs active-low pulse to initiate a conversion on the AD574. The monostable also provides an active-high pulse to latch the digital output of

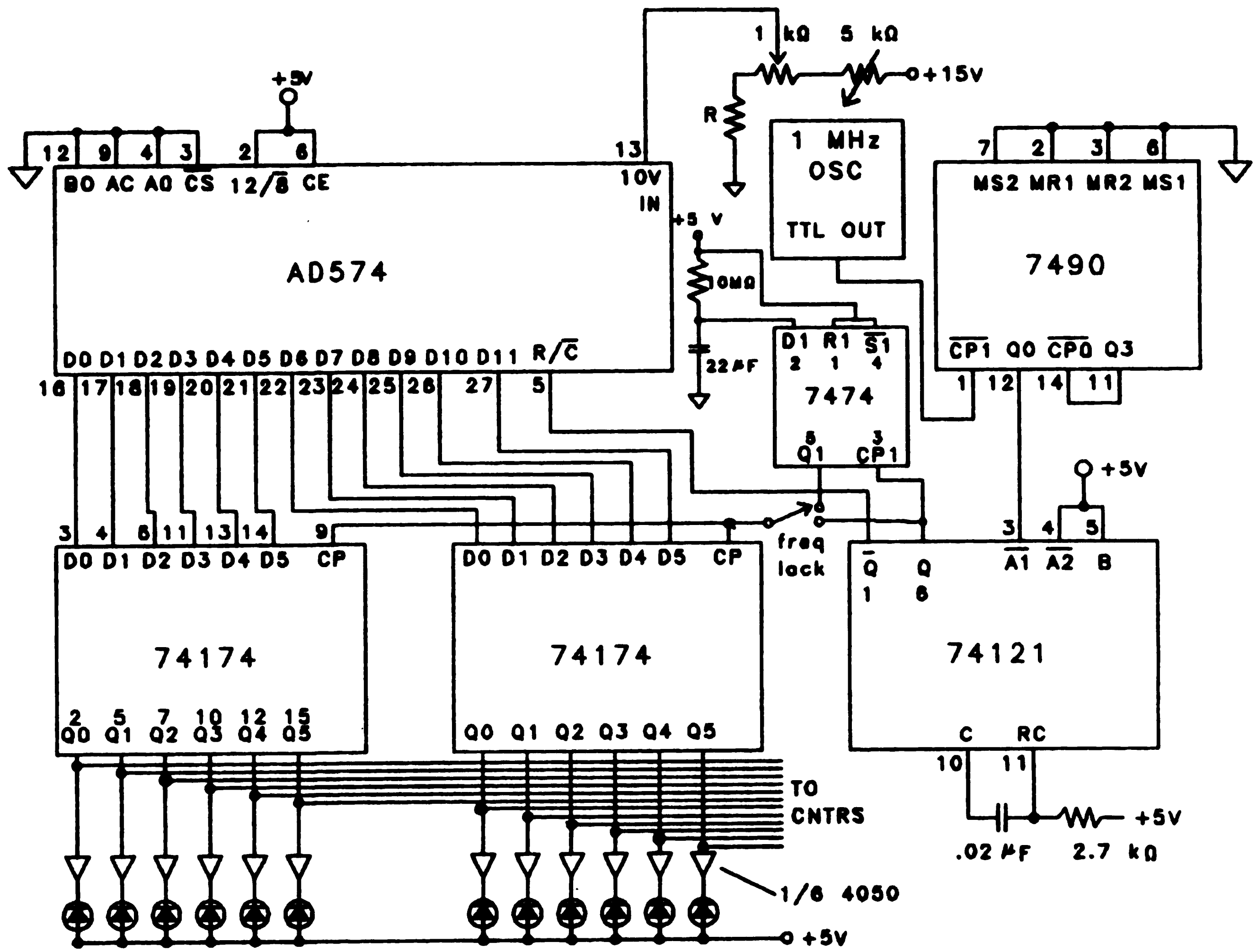


Figure 27. Bimorph frequency selection circuit.

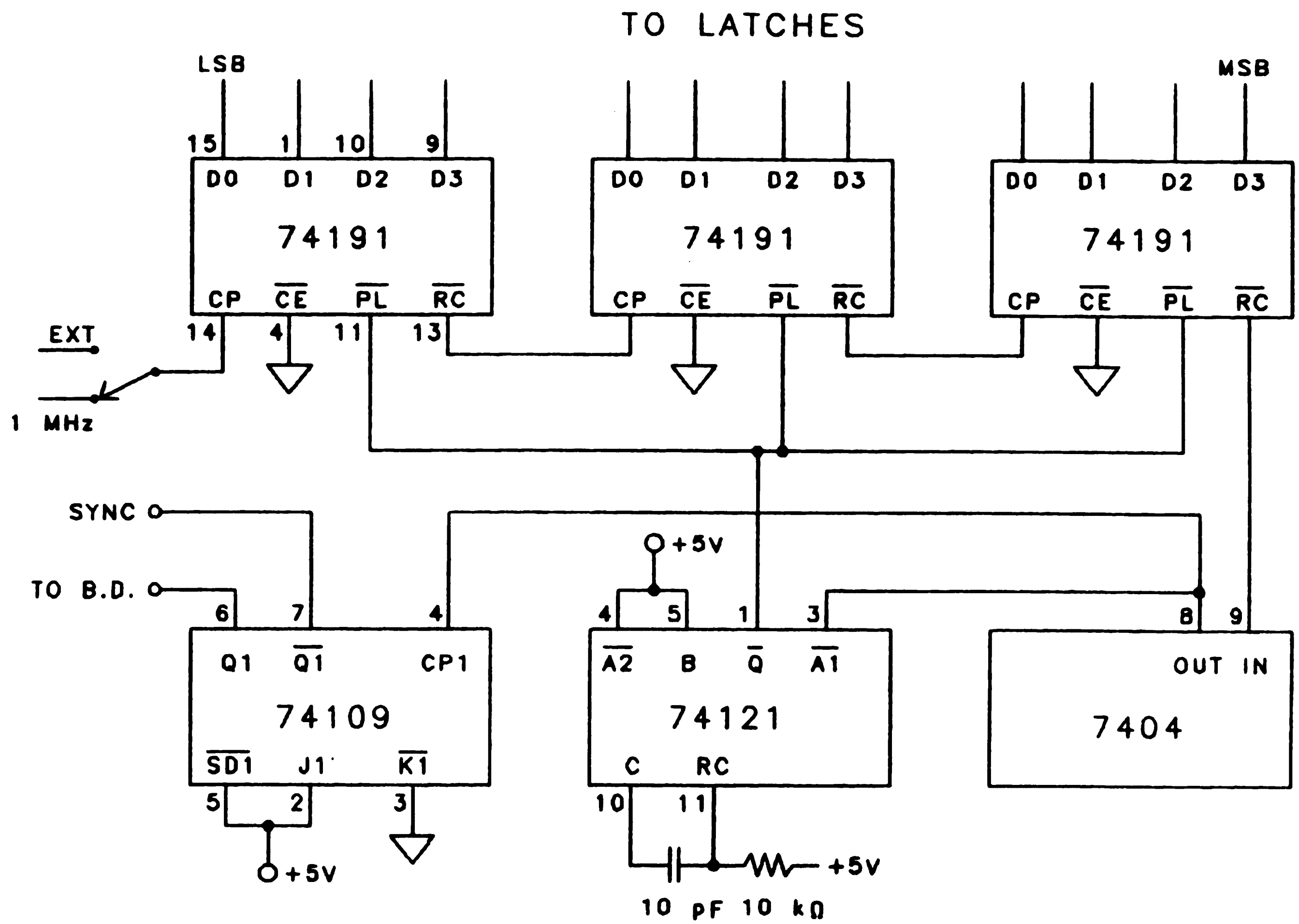


Figure 28. Bimorph frequency divider circuit.

the previous conversion. The latches are connected to both the counter data inputs and to a series of LEDs on the front panel through the 4050 display drivers. The LEDs can be used to determine the current frequency divisor. The frequency is locked in by breaking the connection between the latch pulse monostable and the latches via a front panel toggle switch.

The purpose of the 7474 flip-flop is to provide an initial connection between the latch pulse monostable and the latches should the unit be powered up with the toggle switch in the frequency lock position. This is necessary so that the unit does not initialize with the latches unloaded, which would produce an initial frequency of 500 kHz, too high for the bimorph driver circuit to handle. On power up, the D1 input of the 7474 is at ground potential. The state of D1 is loaded into Q1 on each clock edge at CP1. Eventually, D1 changes state as the capacitor charges, with the result that a single rising edge is sent to the latches with the proper timing relationship governed by the latch pulse monostable. The timing relationships are shown in Figure 29.

Figure 28 shows the part of the circuit concerned with frequency division and waveform output. The input is selectable via a front panel toggle switch between the onboard 1 MHz oscillator and an external input. Three 74191 4-bit binary counters are cascaded by connecting the

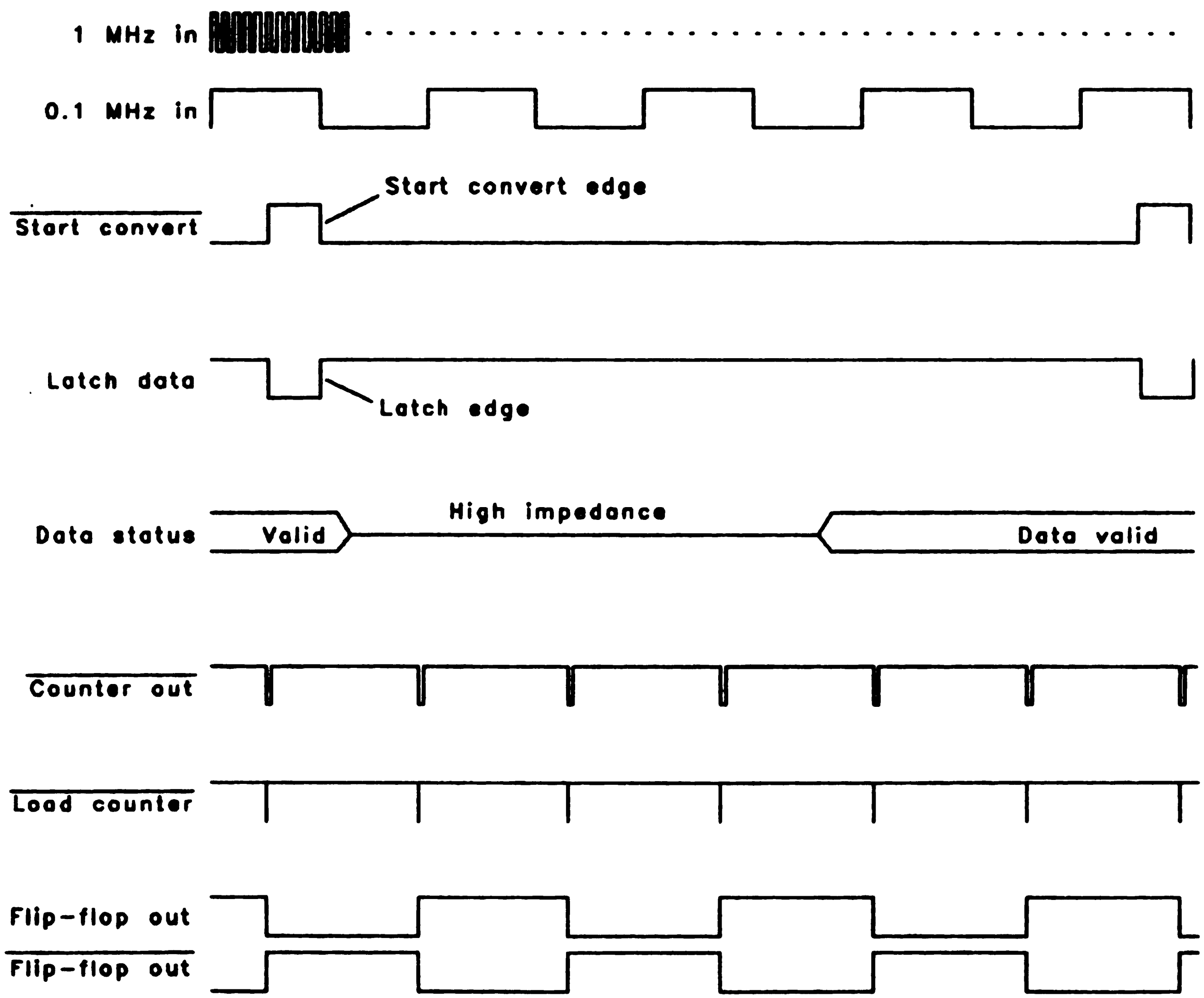


Figure 29. Bimorph frequency selection timing diagram.

active-low rippple count output to the clock pulse input of the next counter. The oscillator is connected to the clock pulse input of the first counter. The output of the last counter is inverted and then connected to both a 74109 flip-flop, which is used to make the output a square wave, and a 74121 monostable multivibrator, which generates a load pulse of approximately 70 ns. When the most significant counter issues a ripple count, the three counters are simultaneously reloaded from the latches to begin the next count cycle. Two outputs are available from the 74109. The non-inverted output is connected to the bimorph driver input. The inverted output is made available at the front panel for synchronization of external devices.

Pulsing Frequency Selection Circuit

The selection of the frequency with which droplets are pulsed out of the main stream is made via three thumbwheel switches mounted on the front panel of the instrument. Because this frequency does not need to be continuously adjustable and the output does not have to be converted to a 50% duty cycle, this circuit, shown in Figure 30, is considerably less complex than the bimorph frequency selection circuit.

Three 74190 4-bit BCD counters are cascaded by connection of the ripple counter output of one counter to the clock pulse input of the next. The input to the first

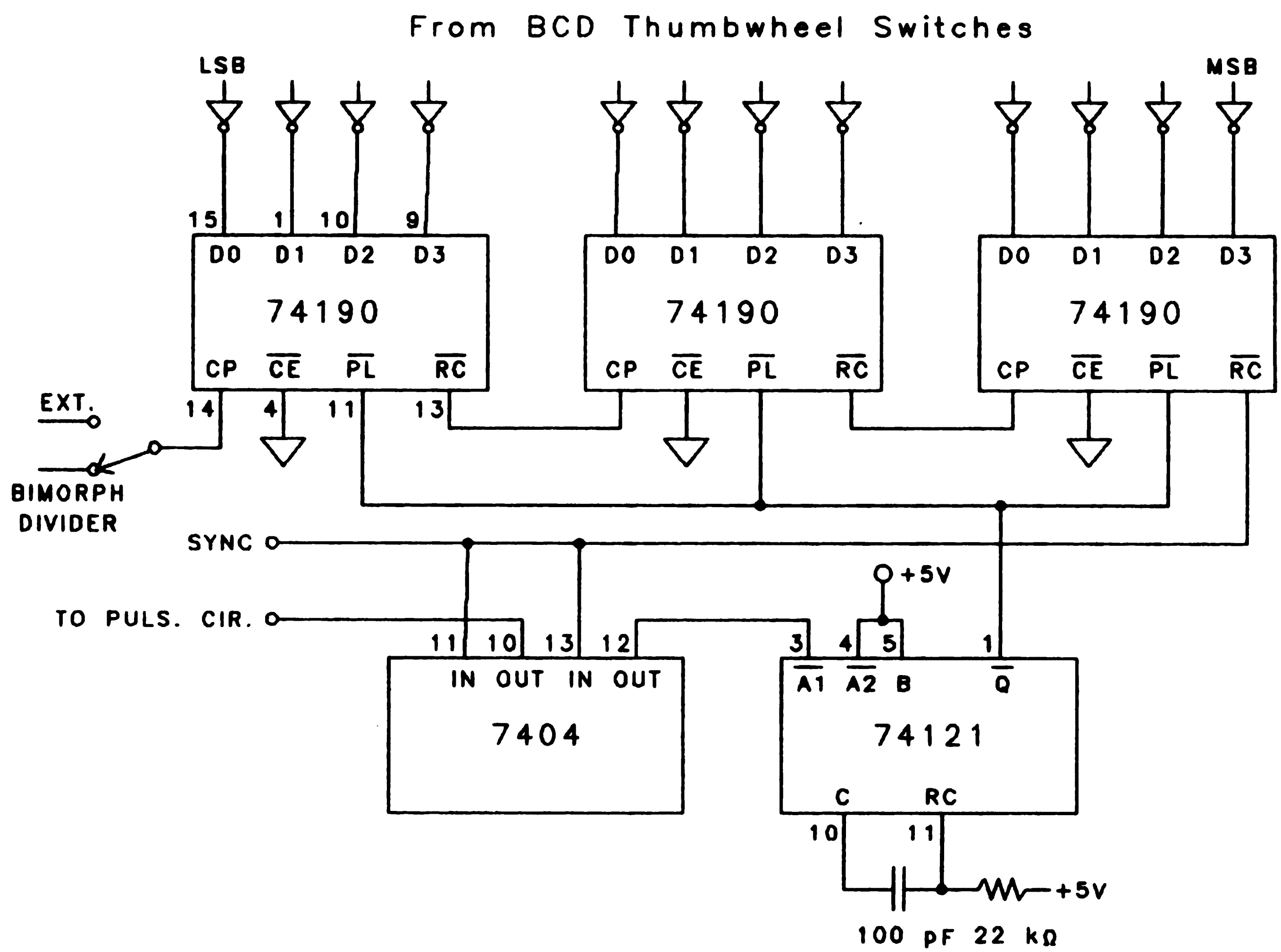


Figure 30. Charging frequency divider circuit.

counter is selectable from a front panel toggle between an external input or the output of the bimorph frequency selection circuit. The counters are loaded from the inverted outputs of the BCD thumbwheel switch when the inverted ripple count of the most significant counter triggers the 74121 monostable multivibrator. The latter issues a load pulse of approximately 1 μs. The output of the most significant counter is also connected to a 7404 inverter, which converts the active-low ripple count to a high pulse which is connected to the droplet charging circuit input. The non-inverted waveform is available at the front panel. The inverted waveform (internal connection) is used to separate charged droplets from the stream. Uncharged droplets can be pulsed out by externally connecting the non-inverted waveform to the external pulsing circuit trigger input. The width of the either pulse is determined by the period of the input clock frequency.

Adjustable 300 Volt Power Supply

To supply the voltage for charging droplets, the 300 V adjustable power supply shown in Figure 31 was constructed. This is a modified version of a design published by Russo et al. (104). A capacitively filtered full-wave rectifier supplies 300 V to pass transistor ECG238 whose resistance controls the voltage at the output. The resistance of the pass transistor is varied by the TL081 amplifier and control

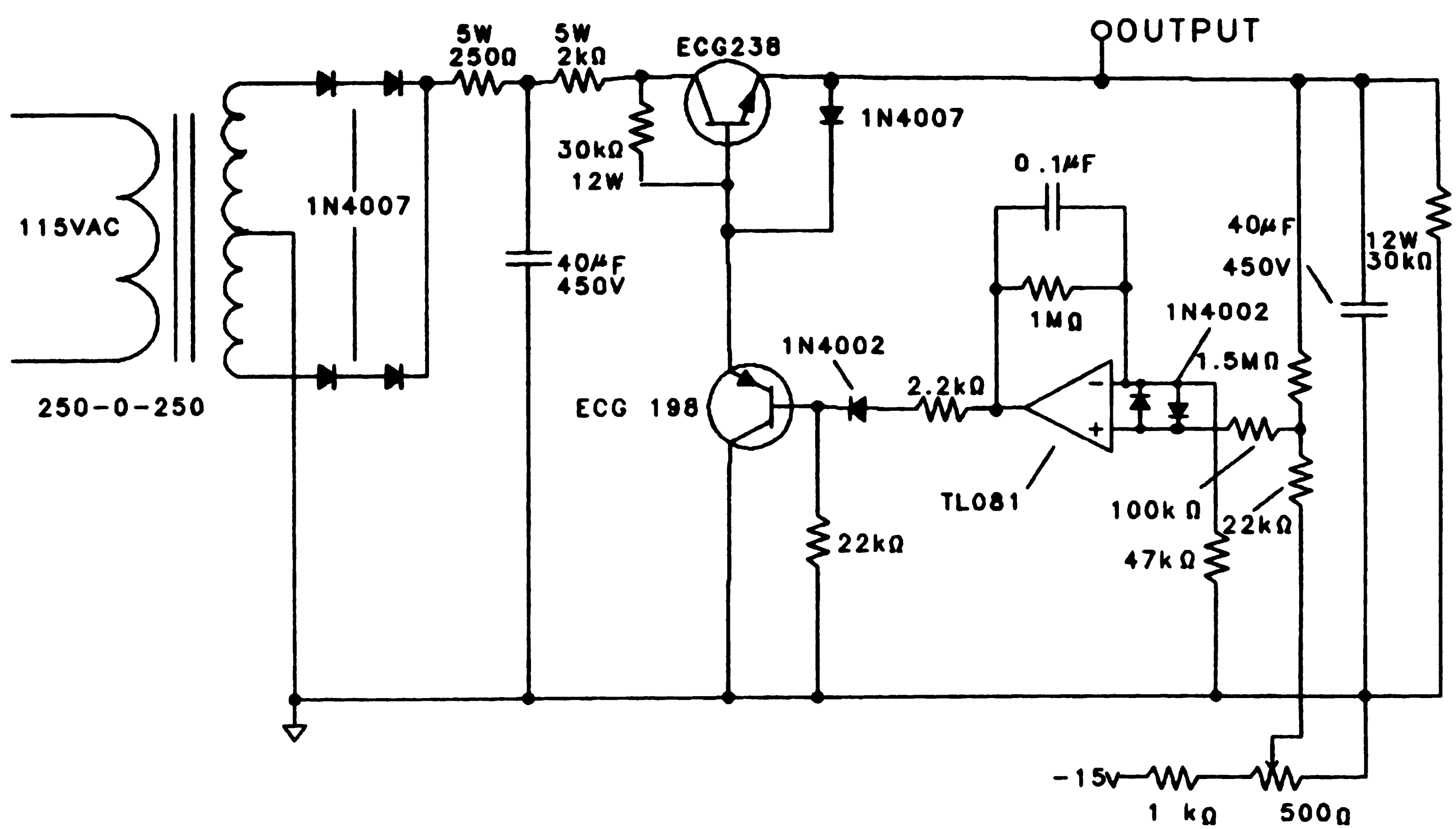


Figure 31. Adjustable high voltage power supply.

transistor ECG198 through the front panel 500 Ω potentiometer. In operation, the voltage presented to the non-inverting input of the amplifier is varied by the front-panel potentiometer. The amplifier corrects for the voltage difference at its inputs through the feedback loop, and the resulting output current varies the resistance of the control transistor. The base current to the pass transistor (and thereby the output voltage) is regulated by the resistance of the control transistor. The particular output voltage selected is displayed on an analog panel meter.

Several modifications were made to the original design. A 250 Ω resistor was added between the rectifying diodes and the filter capacitor to drop the rectifier output voltage slightly, reduce clipping of the AC voltage and give the diodes some resistance to work against. A 1N4007 diode was added at the pass transistor ECG238 to protect it against excessive emitter to base voltage. This transistor is rated for 5 V but it is feasible that a larger voltage could build up if the adjustment potentiometer is turned down too rapidly, or the transformer is turned off. A 40 μF, 450 V capacitor was substituted for a 20 μF, 250 V capacitor on the output for better stability and the higher voltage rating. The 30 kΩ, 12 W load resistor was added for discharging of the output capacitor.

5 Volt Fixed Power Supply

A fairly simple 5 V power supply was constructed as shown in Figure 32. A 12 V peak-to-peak transformer secondary was connected to an encapsulated bridge rectifier. The output was capacitively filtered and regulated. The ripple at a 33 mA load was measured to be < 2 mV or 0.04%. With the digital frequency meter described below connected, the noise increased to about 15 mV, which was considered acceptable performance.

Frequency Meter/10 MHz Frequency Source

A counter/timer/frequency meter (CTFM) was constructed from an ICM7226A evaluation kit (Intersil, Cupertino, CA). The ICM7226 is a fully integrated universal counter and LED display driver. It combines a high frequency oscillator, a decade timebase counter, an 8 decade data counter and logic and driver circuitry to directly control a large 8 digit LED display. The ICM7226 can function as a frequency meter, period meter, frequency ratio counter, time interval counter, or a totalizing counter. It is supplied in the evaluation kit with a 10 MHz crystal oscillator, which is accessible through a buffered oscillator output on the chip.

The CTFM is utilized in the IDG to perform various measurements on the generated waveforms. A front-panel rotary switch allows selection between the outputs of the bimorph or pulsing frequency selection circuits, or the

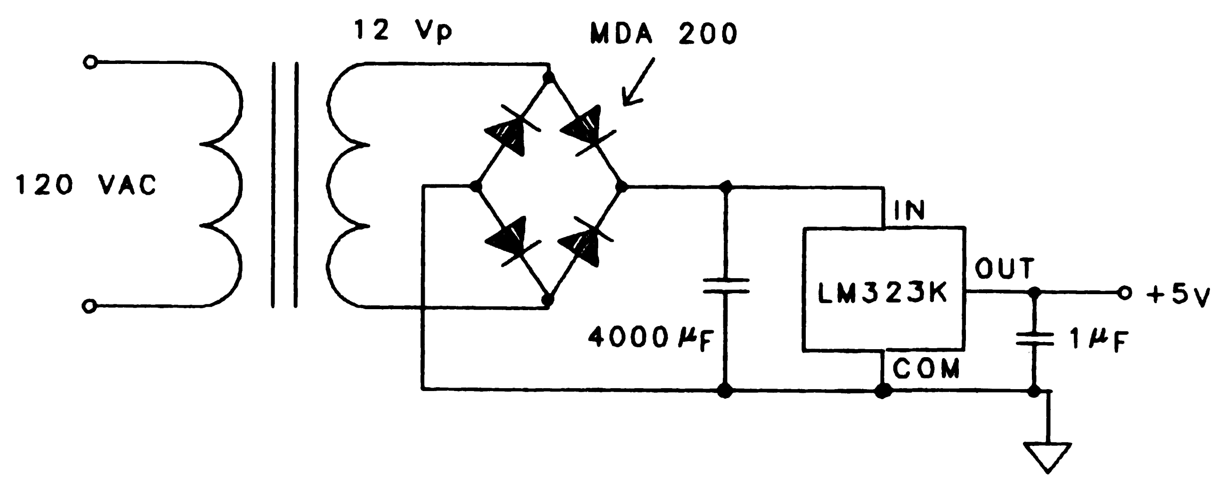


Figure 32. Schematic of 5-volt power supply.

inputs of the bimorph driver or droplet charging circuit. Inputs to the CTFM are buffered with a 7404 inverter. The most common use of the CTFM is for measurement of the droplet production frequency.

The 10 MHz oscillator is also utilized as an external frequency source for the bimorph frequency selection input. The oscillator is buffered in the CTFM and can be connected directly to the external input for the bimorph frequency selection circuit. Care must be taken to ensure that the frequency selector is adjusted with the internal 1 MHz oscillator to a value less than 5 kHz so when the 10 MHz clock is connected the resulting frequency sent to the bimorph driver will be within the proper limits (i.e., less than 50 kHz). This higher clock frequency can be helpful in obtaining more resolution when high droplet production frequencies are used, because higher divisors can be used with the 10 MHz input.

6. Computer-controlled Droplet Generation

The stand-alone version of the IDG is suitable for many applications, but suffers from two distinct drawbacks. First, only a single droplet can be pulsed out of the stream at a time. Second, no provision is made for adjusting the phase between the waveforms used for droplet charging and droplet production. The phase can be adjusted indirectly by decreasing the amplitude of the bimorph driver, which causes the droplets to break off at a different point in time, but this has the unfortunate side effect of decreasing the stability of the droplet stream. Several additional features were desired in the droplet generator as well, especially for use as an HPLC interface. Frequently in HPLC the nature of the mobile phase varies with time. Such variation requires continuous readjustment of the droplet parameters to correct for variations in viscosity and surface tension. A means of making these adjustments automatically would be very advantageous. These problems were solved by placing the IDG under computer control as described below.

Figure 33 contains a block diagram of the IDG configured for computer control of droplet generation. The bimorph driver, pulsing circuit, strobe synchronization circuit and power supplies are identical to those described previously. However, the controlling waveforms for these circuits are now produced externally by the AMD 9513 System Timing Controller (STC) described previously. The reader is referred to part A, section 2 of this chapter for a description of the STC and microcomputer components.

The STC is configured as shown in Figure 34. Three counters were used to generate the waveforms for controlling the IDG. Counter 1 produces the waveform for droplet production and is configured to count down repetitively. A value of one-half the desired bimorph period is loaded into

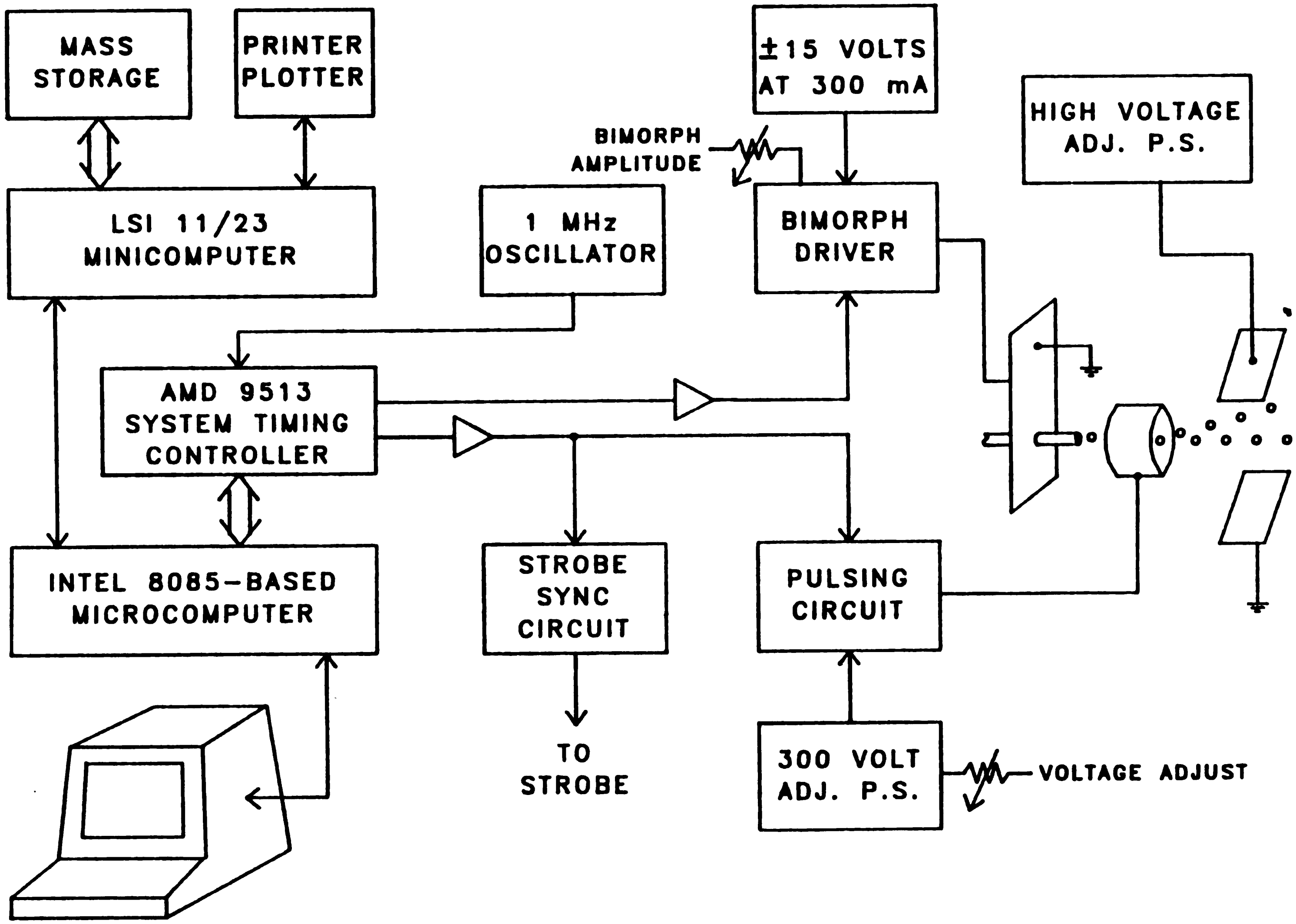


Figure 33. Block diagram of the computer-controlled version of the isolated droplet generator.

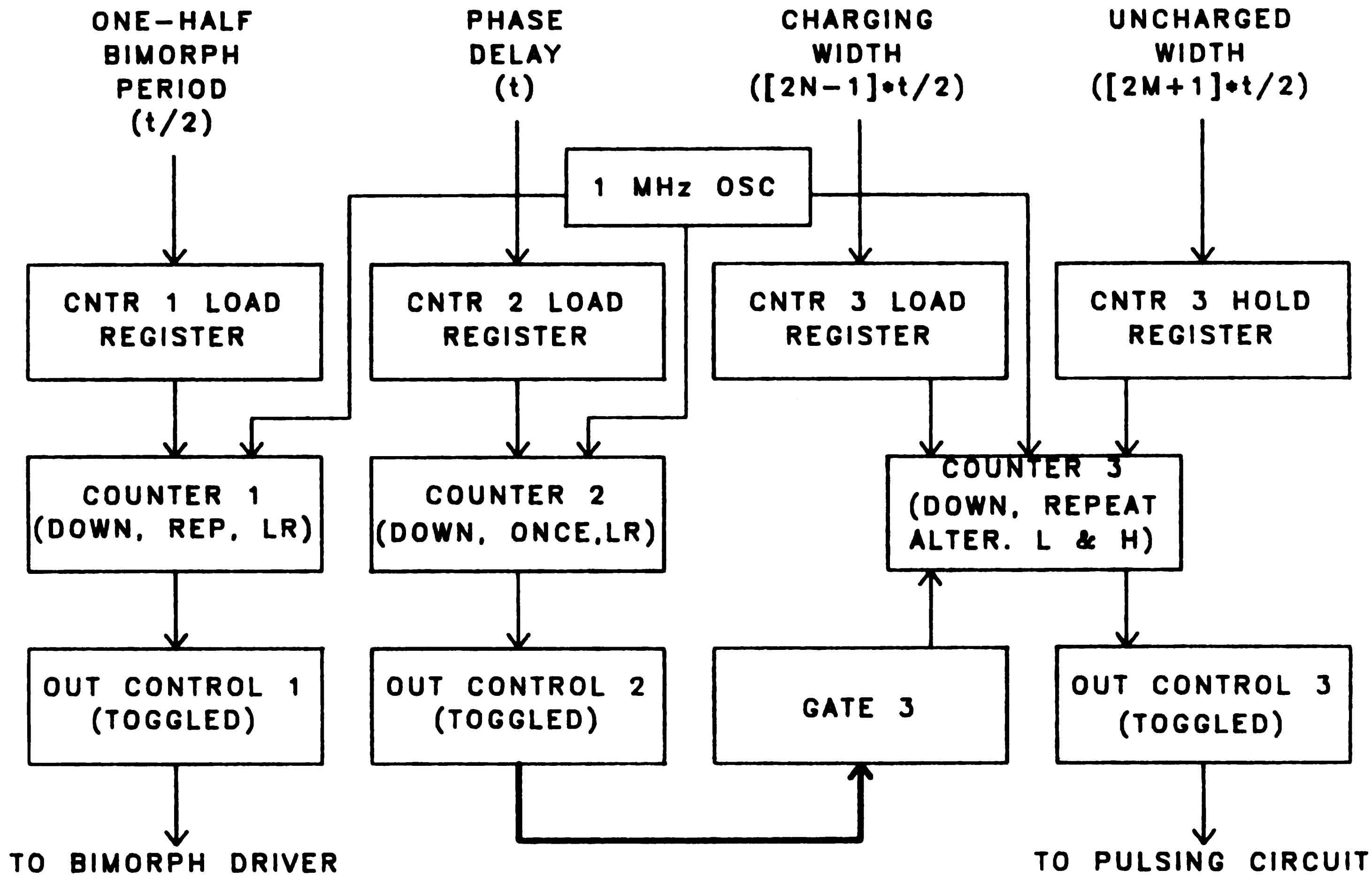


Figure 34. Configuration of the AM9513 system timing controller for isolated droplet generation.

the counter's load register. With the output control enabled, the output of the counter changes state at each terminal count, producing a square wave with a period equal to twice the value in the load register.

The second counter is used to control the phasing between the droplet production waveform and the droplet charging waveform. This counter is also configured to count down, but it only counts once. Upon reaching terminal count, the output changes state. This output is hardwired to the gate of counter three. Counter two is initially loaded with a value equal to the period of the desired droplet production frequency and is adjusted to produce the desired phase change as described in the software chapter.

Counter three is used to produce the waveform for droplet charging. This counter is again configured to count down repetitively, but it alternates the count source between the load and hold registers. The charging width desired is placed in the load register and the uncharged width is placed in the hold register. Initial estimates of these values are calculated in software as shown in Figure 34, where N is the number of drops to be charged and M is the number of drops uncharged. The output control is enabled so that the output changes state at each terminal count. The result is a complex duty cycle waveform with complete control over the charging width, uncharged width, and phase with respect to droplet production.

A comparison of the waveforms produced in the stand-alone mode and the computer-controlled mode are shown in Figure 35. As can be seen from the figure, the waveforms used to control the bimorph driver are identical. The waveform used for droplet charging, however, is much more versatile in the computer-controlled mode.

Since only three of the five counters available on the STC have been used, the other two are available for generation of synchronization signals to control external devices. An example of this is shown in the applications chapter where the firing of a spark discharge was adjusted in phase with respect to the introduction of a droplet. Alternatively, these counters could be used as a real-time clock to make changes in the droplet parameters automatically as a function of time.

A photograph of the droplet generator and frequency meter is shown in Figure 36. The upper portion of the front panel contains status lights and the analog meter for reading droplet charging voltage. The center section of the front panel contains switches and potentiometers for controlling the various droplet generation parameters in the stand-alone mode. Inputs and outputs are located on the bottom portion of the front panel. The bottom photo shows a photograph of the bimorph and electrode mounting assembly.

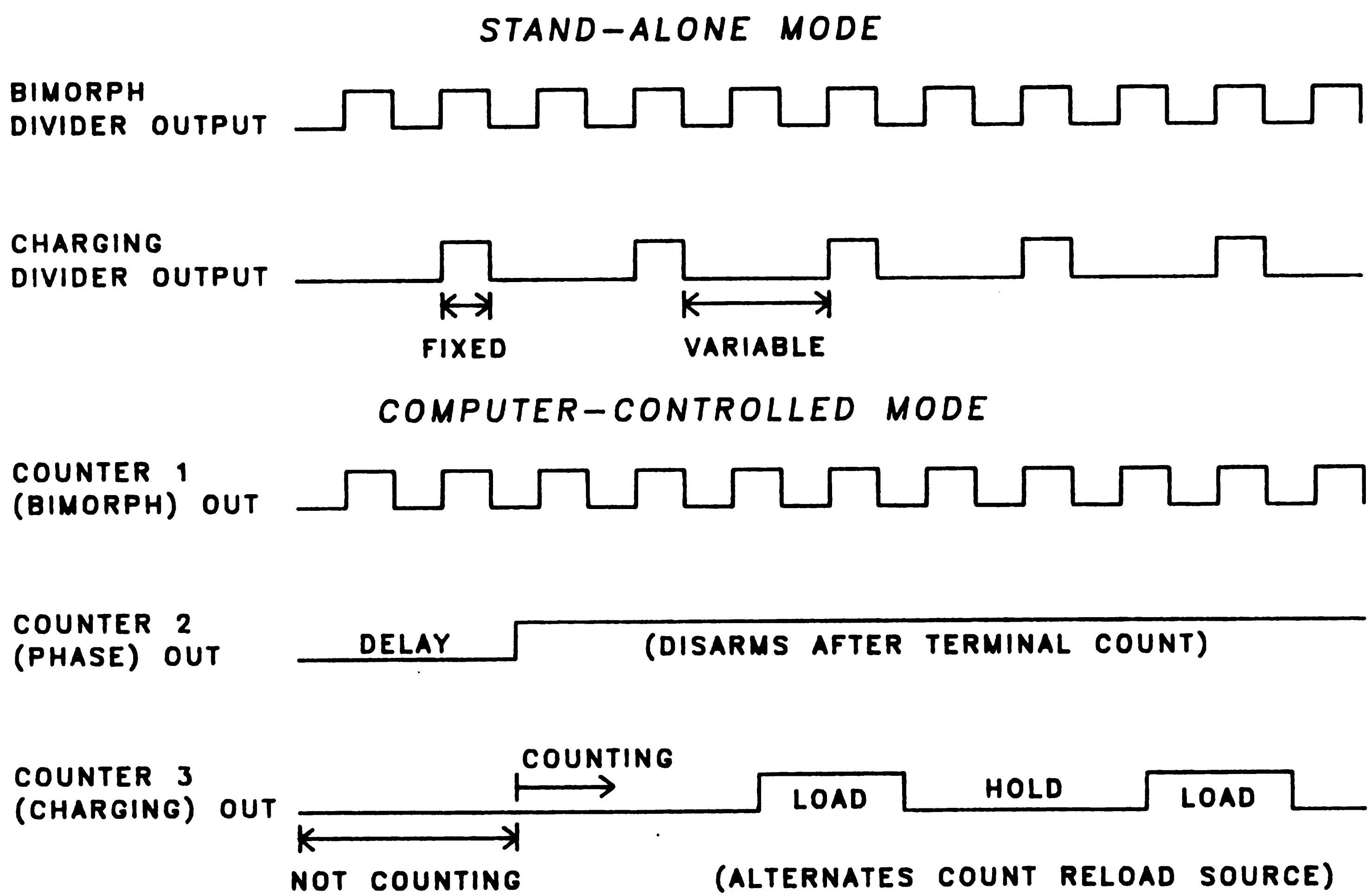
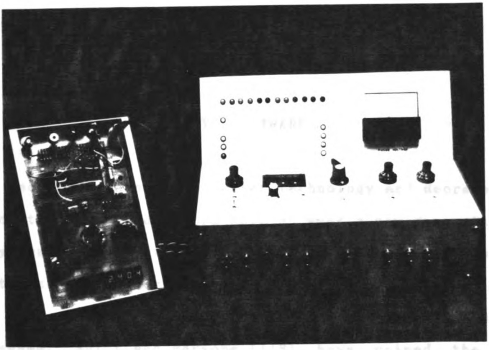


Figure 35. Comparison of timing diagrams for the stand-alone mode and the computer-controlled mode of the isolated droplet generator.



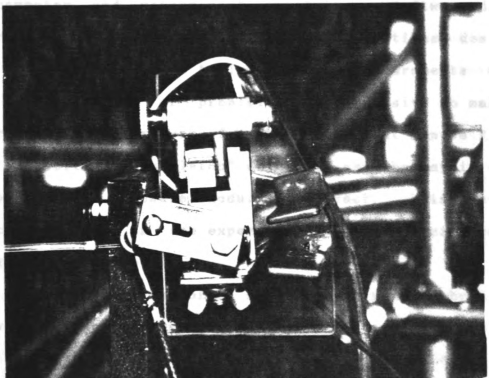


Figure 36. Pictures of the isolated droplet generator (top) and bimorph mount (bottom).

IV. SOFTWARE

Recent advances in computer technology and decreases in the cost of microprocessors have spawned a new generation of analytical instruments. The new instrumentation is designed to utilize one or more microprocessors as an integral part of the control, data acquisition or data reduction processes. Salit and Parsons (118) have coined the term "software-driven instrumentation" to refer to this symbiosis of computer and measurement systems. Software-driven instruments offer many advantages over conventional designs. They offer the analyst a tool for making measurements which may be impossible or prohibitively expensive to make by other means. The hardware for the measurement can be fixed, with a software "skeleton" which defines the way in which the particular hardware modules interact. This permits radical changes in the experiment while minimizing costly changes in hardware.

This chapter explores some of the philosophy of the software-driven instrument. The computing environment and the language requirements are discussed. Specifically, the FORTH operating system and computer language is presented to familiarize the reader with its advantages and disadvantages, especially as related to instrument control. The specific control software for the multidimensional HPLC is presented, followed by a discussion of software for the computer-controlled version of the isolated droplet generator.

A. FORTH Operating System and Programming Language

Successful implementation of a software-driven instrument requires a choice of operating system and programming language. Four requirements are important. First, the user should have a readable command set which requires a minimum of computer expertise to use. Second, the software should be able to be easily modified or appended with user programs without reloading the entire set of routines. Third, the user should still have access to the complete power of the language itself. Finally, the operating system must be compatible with the microcomputer environment.

An operating system based on the FORTH language was well suited to these requirements. FORTH is a programming language and stand-alone operating system with many features which make it ideal for instrument control through mini- or micro-computers. The version implemented here is polyFORTH, which is a multi-tasking version based on the FORTH-79 standard (FORTH, Inc., Hermosa Beach, CA) and installed in the Intel 8085-based microcomputer presented in Chapter 3.

FORTH is structured in a multi-leveled, or threaded, fashion. The standard kernal consists of a dictionary of individual, directly accessible routines for terminal I/O, floppy disk I/O, 16-bit integer math, iteration and flow control, memory I/O, program editing, and interactive compilation. We have also added routines in ROM for communication with the 11/23, and have altered the disk routines from the original floppy-based version to a version that uses the 11/23 as a pseudo-disk. These routines are all available upon power-up.

The kernal definitions (also called words) are used to build higher level definitions, which can also be used to define additional words, ad infinitum, until a set of high-level commands for controlling the instrument is obtained. The additional words can be defined interactively through the terminal, or by loading previously-saved definitions from disk. As new definitions are added to the dictionary, the power of the system increases.

Interactive compilation is one of the most powerful features of the language for instrument control. The user can easily combine commands into an ordered set of control operations without having to re-compile and re-load the entire instrument operating system. For example:

```
:  SPECTRUM 3000 WAVELENGTH 2 ANG/SEC UP 4000 STOPSCAN ;
```

might be the definition of the word "SPECTRUM" which would set a monochromator to 3000 angstroms and scan up at 2 angstroms per second until 4000 angstroms is reached. This definition can be typed directly into the terminal to be compiled immediately, so the experiment can be run without delay. The colon character is used to begin a definition and the semicolon stops compilation.

The arguments to the word can also be taken from the parameter stack. This is one of the major philosophies of FORTH, prefix notation, which allows one to pass parameters conveniently from one routine to another via the parameter stack. This philosophy has also been a criticism of FORTH, but front-end routines can easily be made more user-friendly by defining a word to prompt for number input.

Another feature of polyFORTH is its multi-tasking capability. This enables several background tasks to operate concurrently with the foreground, or terminal task. Each background task has its own parameter stack, but is not capable of terminal I/O. Parameter input to a background task is performed via common variables.

Although FORTH has performed admirably as a control language in our system, there are several valid criticisms. Because only integer mathematics is available, the language is not well-suited to large-scale numerical manipulations.

This problem has been solved by uploading data to the 11/23 minicomputer and performing computation-intensive tasks there with FORTRAN. Documentation and readability can be more difficult in FORTH if precautions are not taken by the programmer. Because a program consists of such small segments of code, it is difficult to follow a particular definition if some segments are unfamiliar to the reader. This can be lessened somewhat if the programmer documents each segment adequately. The FORTH indexing utility described below also helps locate each segment on disk so the code can be referred to easily.

B. MHPLC Operating Routines

The organization of the routines for operation of the multidimensional HPLC is shown in Figure 37. The terminal handler is an operating system task which is active when no foreground word is executing. Its function is to process commands typed to the terminal, decode numbers, and display information. The terminal task can access all levels of definitions, foreground and background, as well as variables and constants.

The event controller is a task which repeatedly checks the real-time clock to see if it is time to pass control to the next routine. Since this is a simple task, additional code has been added within the comparison loop to allow execution of other definitions without permanently stopping

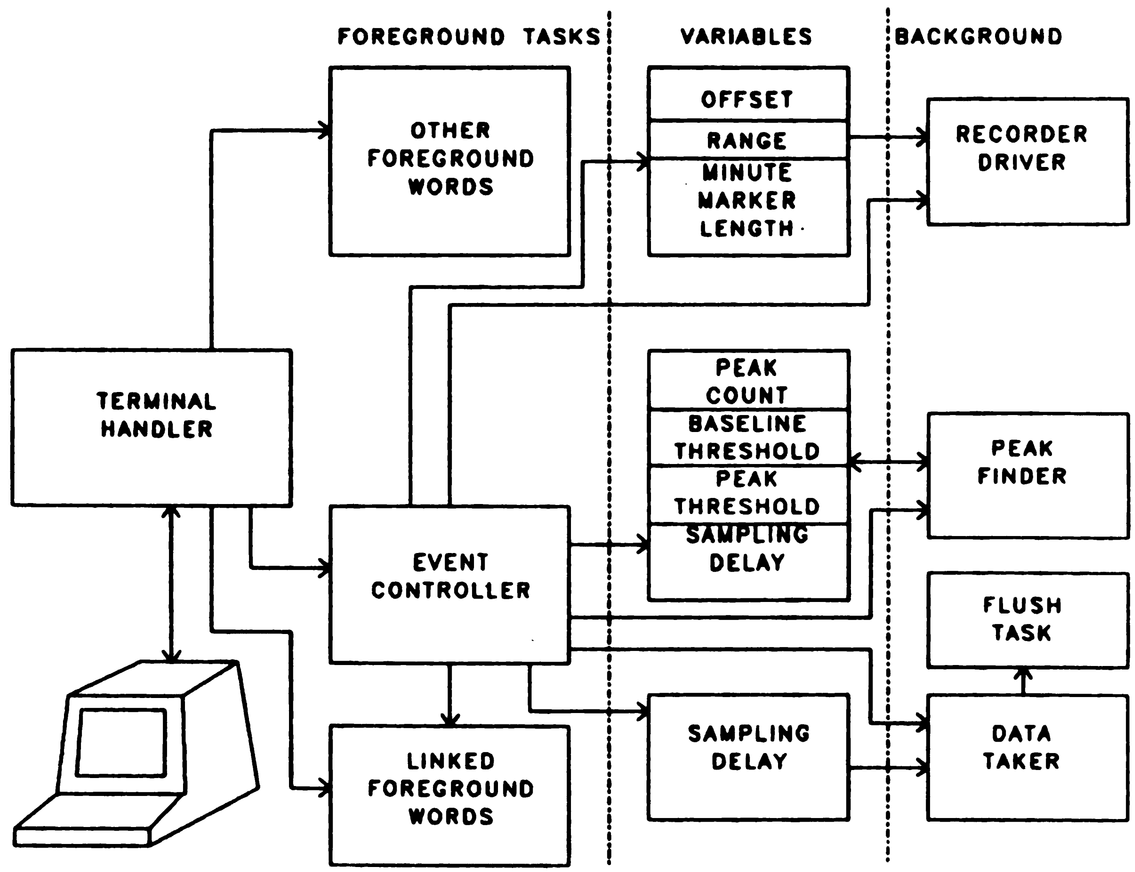


Figure 37. Organization of multidimensional HPLC operating routines.

execution of the event controller routine. This is done by a process called vectored execution and is discussed in more detail below. Four independent background tasks are defined, three of which can be activated directly or through the event controller. Parameters are passed to and from the background tasks through the variables shown.

The operating routines for the MHPLC can be divided into the four categories of SP8700 control, valve control, data acquisition and event control. Of course, all kernal definitions and user definitions not directly related to instrument control are also accessible to the user. The kernal routines are too lengthy to be discussed here. The reader is referred to one of two published texts, "Starting FORTH" (119), or "Using FORTH" (120). The more important user definitions are discussed below.

1. Control of the SP8700 Solvent Delivery System

The SP8700 is controlled by simulating keystrokes with the keypad emulator circuit discussed previously. The keystroke is initiated by writing a particular bit pattern representative of the key to PIO port A. Programming the control codes has been simplified by defining constants with mnemonics representative of the keys that place the desired control code on the stack. Where possible, the mnemonic consists of the first four letters of the key name. If the key name is two words, the mnemonic consists of the first two letters of each word. Number keys are preceeded with a pound sign to distinguish them from actual numbers. A list of the mnemonics are contained in Table 6.

Table 6. Key Code Definitions.

Key	Mnemonic	Key	Mnemonic
Change Time	CHTI	Test	TEST
Clear Entry	CLEN	Time	TIME
Continue	CONT	1	#1
Display	DISP	2	#2
Delete Line	DELI	3	#3
Edit File	EDFI	4	#4
Edit Mode/Run Mode	EDRU	5	#5
Enter	ENTE	6	#6
Flow	FLOW	7	#7
Hold	HOLD	8	#8
Initialize	INIT	9	#9
Max. Pressure	MAPR	0	#0
Pressure	PRES	.	#.
Purge	PURG	%A	%A
Run Gradient	RUGR	%B	%B
Store File	STFI	%C	%C
Stop	STP	(down arrow)	\/

The key codes are used in conjunction with one of the words PRESS or ENTER. PRESS takes one key code off the stack, depresses the key, and waits for an acknowledgement from the SP8700. If the keystroke is not acknowledged, all foreground tasks are aborted and control is returned to the terminal handler. ENTER assumes all numbers on the stack are key codes and PRESSes them in a first in, first out manner. When done, the "Enter" key is pressed. This word is useful for entering any series of keystrokes where the last key is normally an "Enter" key (e.g., solvent percentage, flow rate, etc.).

Because numbers are a special case, three words have been designed to facilitate pressing number keys. The first, #>KEYS converts a number on the stack to the key codes that represent that number. For example, 1.55 would be converted to the #1 #. #5 #5 series of key codes. GET# accepts up to a 6 digit number from the terminal and converts the number to key codes. The most useful word is ENTER# which functions like ENTER except that it first converts the top number on the stack to key codes.

Another special case is the "Edit Mode/Run Mode" key. Depression of this key toggles the instrument from one mode to the other. Because it is often desirable to switch to a particular mode regardless of what the previous state is, the words EDITMODE and RUNMODE were written for this purpose. This is accomplished by depressing the scroll button, which is used for viewing an SP8700 entry line and thus is not acknowledged in the run mode. If an acknowledge signal is received, the instrument must be in the edit mode and the appropriate action can be taken.

Two SP8700 status words have also been defined. FLOW? delays until the "Flow Ready" light is on. CAM waits until the "Cam Marker" light flashes. CAM is useful for synchronizing with the clock in the SP8700 and for coordinating flow rate changes.

2. Valve Control

Two words control valve actuation and sensing. CCW turns the valve whose number is on the stack in the counterclockwise direction. If the valve is already in the counterclockwise position, this word has no effect. Otherwise, the valve position is sensed after a short interval to determine if the valve change was successful. If the valve does not switch within the appropriate interval, all foreground routines are aborted and control is returned to the terminal handler. CW is the equivalent word for turning a valve in the clockwise direction. Valve numbers 1 or 2 are appropriate arguments.

3. Data Acquisition and Storage

This routine operates as an independent background task. It is initiated by the word TAKEDATA. Acquisition can be temporarily halted by the word SUSPEND and resumed by the word MOREDATA. A data taking run is ended with the word ENDDATA. The data taking rate is controlled by the variable 'REST, which contains the number of hundreths of seconds between points. The delay is performed by the AM9513 STC, and is thus independent of software activity. Data are stored as time-data point pairs, where the time is the number of tenths of seconds since injection and the data point is a 12-bit number stored in a 16-bit cell. These words can all be accessed from the event controller so changes can be performed while a run is in progress.

The data are stored on the 11/23 in the FORTH pseudo-disk file DFLTDAT.FTH. The size of this file determines the number of data points which can be taken. The current default is 50 blocks which corresponds to 12,800 data points. The default sampling delay is 0.12 seconds. The easiest way for the user to set the appropriate delay is to use the word LENGTH, which prompts for the expected length of the chromatogram in minutes. The delay is then set to the rate which will fill approximately 80% of the available file space.

A single data-taking run proceeds as follows. The I/O buffers are flushed, and the pseudo-disk file name is changed from the program file to the data file. Data taking is initiated at the rate specified, and the data are stored in the I/O buffer. When 256 points are taken (the capacity of the buffer) data storage is switched to the second buffer, and the data acquisition task starts the flush task to send the full buffer to the data file. In the event that the second buffer becomes full before the first buffer is finished being flushed, data taking is temporarily halted. This has never happened, but is feasible if the 11/23 is exceptionally busy.

If data taking is SUSPENDed, the acquisition task is halted, but the state of the task is preserved. When acquisition is re-started with MOREDATA, a flag is stored in the data buffer consisting of a -1 followed by the current value of 'REST. The flag is not used directly, but is simply a comment noting the change in sampling interval.

When acquisition is terminated with ENDDATA, both buffers are flushed, the acquisition task is halted, and the user is asked for a file name. The number of blocks acquired is then transferred to the new file, and the disk file specification is changed back to the program file (MICRO.FTH).

4. Peak Finding Routine

This routine is an independent background task whose function is to detect a chromatographic peak and make an attempt to measure the peak height in real time. Three parameters control peak sensing: the sampling interval 'TIME (100ths of second units), baseline threshold 1THRESH (in ADC units) and peak maximum threshold 2THRESH (also in ADC units). These values may be changed directly by altering the above variables, through the prompting words !THRESH and SETTIME, or through the event controller.

The start of a peak is sensed by comparing the difference between the first two data points to 1THRESH. If the difference is less, another data point is taken, and the second and third is compared and so on. If the difference is greater than 1THRESH, the start of a peak is assumed, and the routine begins watching for the peak maximum. The maximum is determined by comparing the difference of two subsequent data points to 2THRESH. If the difference is greater than 2THRESH, and in the downward direction, then the first data point is assumed to be the peak maximum. A report flag is then set for the event controller to process. The report contains the time of peak start, time of peak maximum, peak height, and baseline value.

The peakfinder also maintains a counter, #PEAKS, which is updated each time a peak is recognized. This count can be used as a basis for event control. For example, a valve could be switched after the 5th peak has been recognized. Since sometimes a sample contains more or fewer peaks than expected, the counter can also be incremented or decremented or zeroed through the event controller or by the respective words I#PEAKS, D#PEAKS, and Z#PEAKS. In this manner, even if a sample is not well-behaved, a run can be salvaged by manual correction.

The peak recognition algorithm is not perfect in that as the sampling interval is decreased, the baseline value is determined to be further up the beginning of the peak. This can be reduced to some extent by lengthening the sampling interval, but this occurs at the expense of properly sensing the peak maximum. The default values of 20, 40, and 50 for 1THRESH, 2THRESH, and 'TIME, respectively, do a fairly good job for well behaved chromatograms with fairly sharp peaks.

The peak maximum and time of peak maximum, which are of the most value to the chromatographer, are reported quite accurately. The baseline value should be checked manually by the word ?SYS, which issues a system status report including the current ADC value.

5. Event Control

This classification is probably the most versatile group of words for creative MHPLC control. Events such as valve switching, solvent change, or flow change can be based on time, HPLC status, detector output or any combination thereof.

Time-based Event Control

Time-based commands include EVENT, the event controller which waits until the specified time after injection, and WAIT, which is an absolute delay. WAIT takes an argument off the stack (in hundreths of seconds) and simply delays for that time interval. EVENT takes two arguments off the stack, minutes and seconds, and delays until the real-time clock exceeds that value. Normally this is the time from the injection, where the clock is automatically started by sensing the position of the injection valve. This is accomplished with the word INJECT. SYNC is a similar word, except it prompts for injection at the appropriate time for synchronizing the microcomputer clock with the clock in the SP8700. Both clocks are then started. The microcomputer clock can also be started without injection by use of the word CLOCK.

EVENT is a very powerful word which allows access to a considerable amount of the operating system during a run. It is also responsible for processing the peakfinder report and displaying the present time and time of the next event. EVENT uses single-letter commands to pass control to a word through a process called vectored execution. Specifically, each letter is used as an offset into a table of addresses. The address is then used by FORTH to determine where the word resides in the dictionary. In use, a key is struck once, to get the event controller's attention, and then the key representing the word desired is struck. A list of the keys and their corresponding words is contained in Table 7.

Detector-based Event Control

Event sequencing based on detector output is perhaps the most powerful method of control. Timing is very crucial in column-switching applications, and often HPLC retention times are not very reproducible from run to run. When limited to simple time-based sequencing, frequent timing adjustments are often needed. If detector output is used as a basis for decision-making, programming can be simplified and erroneous runs minimized.

Several commands are available for detector-based sequencing. The peak count, as mentioned above, can be used

Table 7. Event Controller Commands

Key	Word	Function
A	ABORTRUN	Abort run, return control to term. handler
B	TAKEDATA	Begin taking data.
C	CLR	Clear screen.
D	SETTIME	Set delay for peak finder.
E	ENDDATA	End a data taking run.
F	D#PEAKS	False peak, decrement peak count.
H	EHELP	Lists event controller commands.
I	I#PEAKS	Increment peak count.
K	Z#PEAKS	Kill (zero) peak count.
L	LMARK	Sets length of minute marker for recorder.
M	MOREDATA	Restarts data taking after a SUSPEND.
N	NEWREST	New data taking interval.
O	1STOP	Turns off recorder driver task.
P	?PEAK	Turns on peak finder task.
Q	4STOP	Turns off peak finder task.
R	DIGREC	Turns on recorder driver task.
S	?SYS	Displays system status.
T	!THRESH	Sets peak finder thresholds.
U	SUSPEND	Suspend data taking temporarily.
V	VIEW	List a block on the terminal.
X	TOGGLE	Stop/Restart the microcomputer clock.
Z	ZERO	Sets recorder zero for recorder driver task.

in conditional execution. The command >%FS or <%FS will delay until the signal is greater or less than the specified percent of full scale recorder deflection. These commands are very useful for heartcutting. The relative counterpart to these commands is %FSCHG. This command delays until the signal changes by the specified percentage of full scale, regardless of the direction of the change. The commands >A/D, <A/D, and A/DCHG are similar, except these take ADC units as arguments.

To assist in programming with detector-based event control, ten registers have been established which can be used to save and recall the ADC values corresponding to detector output. The word SAVE takes a number from 0 to 9 off the stack and saves the current detector output in the specified register. RECALL takes the register number off the stack and replaces it with the previously saved detector value.

Most applications use combinations of event control commands, linked in either a logical AND mode or a logical OR mode. FORTH allows easy implementation of either combination. For example, one may want to wait until six minutes after injection, wait for two peaks, wait for baseline, then switch a valve after a 10% change in the signal, which might be the rising edge of the next peak. With this type of strategy, very powerful and inteligent event sequencing can be accomplished.

6. Recorder Control

The strip chart recorder is used both as a method of obtaining real-time output and as a means of re-plotting a previously stored data file. This method of graphics output produces lower quality plots than can be obtained by using a graphics printer, but it is considerably easier and faster. The chart motor drive can be started or stopped with the words ON and OFF. The chart servo drive is controlled as described below.

Digital Recorder Driver

The digital recorder driver is an independent background task whose purpose is to convert the ADC output to a form compatible with the DAC output connected to the chart recorder input. The task is activated with the word DIGREC, or can be started through the event controller. The output scale is controlled by the two variables OFSET and RANGE, which are conveniently set using the word ZERO. ZERO prompts for the percent of the recorder scale which should be designated zero volts from the detector. The ADC value is then transformed to DAC units according to the equations:

```
OFSET = 2048 - (2048 x % zero input) / 100
RANGE = 4096 - OFSET
DACunits = ((ADCunits - OFSET) x 256) / RANGE
```

If the result is less than zero or greater than 256, the DAC is set to the respective extremes. This method of conversion always retains the complete positive range of the ADC, but allows the user to select what portion of the negative range is displayed on the recorder.

The recorder driver also places a tick-mark on the output every time the real-time clock turns over to the next minute. This allows easy estimation of retention times. This option can be disabled by setting the length of the minute marker to zero using the word LMARK. The default length is 10 DAC units.

The word EVENT makes a similar mark upon initiation. In normal use, this allows the user to see where the injection point is and where each event occurs if the event is immediately followed by another EVENT cycle. The length of this mark can also be controlled by the user by changing the variable EMARK.

Regraphing Utility

The regraphing utility allows one to output a stored data file to the recorder with different scale factors. The output occurs at ten times the speed with which the data were taken, so this is a quick and easy method of displaying a data file. The user sets the chart recorder speed to a factor which is ten times faster than the original to obtain a scale factor of one in the x direction.

The scale factor in the y direction is determined by answering the prompts for the minimum and maximum ADC values to graph. The complete range of the ADC is displayed by using values of 0 and 4095, respectively. Higher maximum values will compress the x output. A higher minimum and lower maximum cause the x output to be expanded.

The regraphing utility is initiated by the word REGRAPH. A file name is prompted for and the file is copied into the default data file DFLTDAT.FTH. The number of blocks transferred is displayed along with any error messages. If the file is not present, or any other file error occurs, the utility exits, and control is returned to the terminal handler.

7. Other Useful MHPLC Words

Several other words have been defined for checking the status of the system. ?SYS was designed to display the status of the background tasks and their controlling variables. It also reports valve positions, SP8700 flow ready and cam signals, and current ADC and DAC outputs. @DAT is the fundamental word used for initiating a single data conversion and placing it on the stack. RUN will continuously display the ADC output until a key is hit. TFI and <TFI> return the time from injection, or since the clock was last started, in units of seconds and tenths of seconds, respectively. RESET can be used to reprogram the AM9513, and SETUP can be used to reprogram the 8255 PIO for use in the MHPLC system. Both of these words are executed when the software is first loaded, however, and do not necessarily need to be used again.

C. Computer-controlled IDG Operating Routines

The software for operation of the IDG in the computer-controlled mode is mostly concerned with programming the AM9513 system timing controller. Figure 38

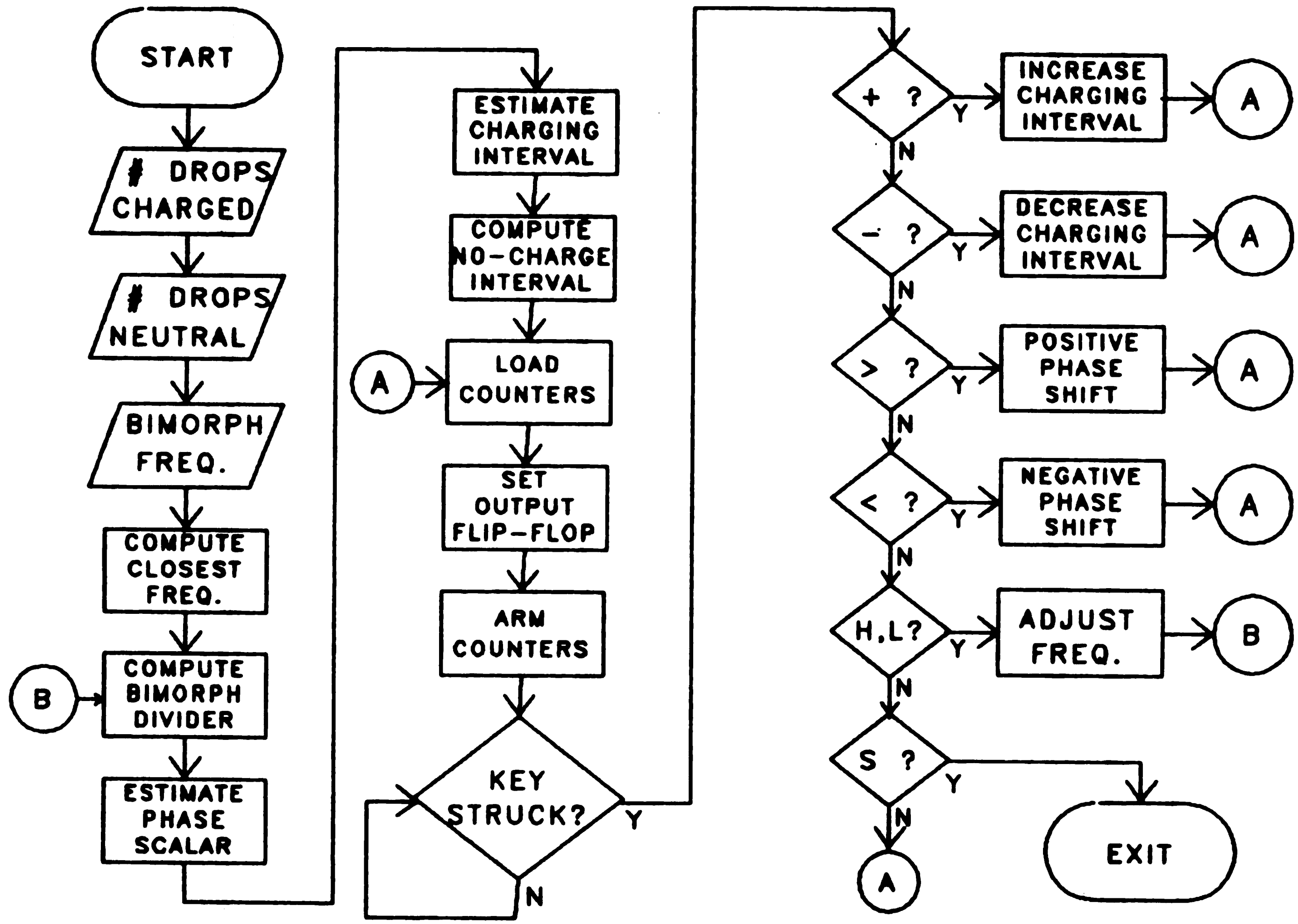


Figure 38. Isolated droplet generator controller flowchart.

illustrates the use of the word IDG, which is used to operate the droplet generator in the repetitious droplet production mode. The initial arguments placed on the stack are the number of drops to be charged, the number of drops uncharged and the frequency in kHz to two decimal places. The program first computes the closest frequency available from the 1 MHz oscillator in the AM9513. An upper frequency limit of 50 kHz is set in software. If the desired frequency is less than this upper limit, the appropriate frequency divider is calculated to be placed in counter 1. This frequency divider is 1/2 the period of the desired square wave.

The phase scalar is then estimated by simply doubling the value used for the bimorph frequency divider. The charging and neutral widths are calculated according to the equations:

$$CW = (2N - 1) \times t/2$$
$$NW = (2M + 1) \times t/2$$

where t is the period of the bimorph frequency, N is the number of droplets to be charged, and M is the number of droplets left uncharged.

After estimation of the proper frequency dividers and pulse widths, the counters are loaded, and the output bits on the AM9513 are set to the correct state. The counters are then armed, and the program goes into an interactive adjustment loop. In this loop, the unshifted + (ASCII 61.) and - (45.) keys are used to adjust the charging and neutral widths. This is done by adding (subtracting) one microsecond from the charging width and adding it to the neutral width, then restarting the counters. Thus, this action does not affect the phasing or frequency of the charging waveform, only the duty cycle. The unshifted < (ASCII 44.) and > (46.) keys are used to adjust the phasing between the charging and bimorph waveforms. This is done by adjusting the phase scalar by one microsecond and restarting the counters. The H (ASCII 72.) and L (76.) keys are used to adjust the frequency of droplet production. This is accomplished by adding or subtracting one microsecond from the bimorph divider, re-estimating the required pulse widths and phase scalar, and then restarting the counters. The S key (ASCII 83.) exits the IDG adjustment task and returns control to the terminal handler. The IDG will keep producing droplets as defined by the last adjustment command.

A second word which is similar to the above is RAMPIDG, which is useful for locating the proper droplet production frequency. This word uses IDG to set an initial upper frequency and then ramps down by adding one microsecond to the bimorph divider at a rate of 1 Hz. The operator can watch the droplet stream until a good production frequency is found, and then a key is pressed. The routine then goes into the frequency adjustment loop as described above. The ramp is continued by pressing the S key. This routine does not automatically adjust for the pulse widths, however, so the pulse voltage should be turned off for the best results.

Operation of the IDG in the single-shot mode is accomplished with the word SINGLE-SHOT. This word takes as arguments a delay in hundreths of seconds. It is meant to be used immediately following IDG. Droplet production and pulsing parameters are set in the normal manner with IDG, and then IDG is exited. When SINGLE-SHOT is invoked, the specified delay is performed in software, and the counters for droplet production are started with the values for the bimorph frequency, phase scalar, and pulse widths that were set with IDG. In this case, however, counter three is reprogrammed to count only once instead of repetitively; thus, only a single packet of droplets are produced.

D. Support Software and Data Conversion

Several support programs have been written to run on the 11/23 minicomputer and are briefly mentioned below.

1. FORTH Support Programs

As mentioned previously, the version of FORTH implemented herein does not use floppy disks for data and program storage, but rather, uses the LSI 11/23 as a pseudo-disk. The program which emulates the FORTH disk is called FORTHPIP and was written by Phil Hoffman (120). It serves to transfer data between FORTH devices, standard FILES-11 format RSX files, RSX FORTH emulator pseudo-disk files, and serial data transmission lines. In this case, FORTHPIP functions in three capacities. First, it sends and receives data between the microcomputer serial line and the emulator file. This file is structured in 1024-byte segments called blocks. This is also the size of an I/O buffer on the microcomputer. Second, it generates hard copy of the programs contained in the emulator file by transferring data to the printer. The third function is to act as a translation program to convert data stored in the FORTH emulator file format to a FILES-11 format that can be accessed by FORTRAN programs and word processors.

Use of FORTHPIP to generate hard copy is rather cumbersome, since a rather long command line is necessary, and the position of the block on a page must be previously known. A short command file called FTHPRT.CMD was written to make updating of FORTH listings easier. This file accepts a block number as input, determines what two other blocks need to be listed on that page, and generates the appropriate command line to print this triad of blocks.

Another useful utility was written to help locate the block in which a word was defined. This utility is a collection of programs run from the command file FTHINDEX.CMD. When run, the utility extracts words defined with :, CODE, CREATE, VARIABLE, and CONSTANT, along with their block number. The words are then sorted alphanumerically and printed to provide a convenient index to a FORTH program file.

2. Data Conversion Software

Graphics output or data reduction is performed by processing the data with the FORTRAN program CRUNCH and then using a graphics package such as MULPLT to obtain high-quality graphics output. The purpose of CRUNCH is to convert a data file stored in the unformatted FILES-11 compatible form produced by FORTHPIP to a formatted ASCII file containing records of the form:

RD time,data

with one record per line. This form was chosen to be compatible with MULPLT and many of the other programs written for data processing on the LSI 11/23 system. CRUNCH does not perform scaling, smoothing or any other manipulations which would alter the original data. These functions are left to the user. Typically data files are CRUNCHed only when necessary, as the formatted file takes up considerably more room than the unformatted file. Data are stored in the unformatted FILES-11 form produced at the time a data collection run is terminated.

V. EVALUATION AND APPLICATIONS

This chapter is concerned with evaluating the performance of the previously described instrumentation. Several studies dealing with band broadening, baseline disturbances, backflushing, and detector-based valve switching are presented. Subsequently, applications pertaining to fuels separations are shown. These separations were performed in conjunction with M.R. Danna (122), and the reader is referred to her dissertation for additional information. Several miscellaneous applications not directly related to MHPLC are also presented.

The isolated droplet generator is also evaluated and sufficient fundamental studies are shown to provide a good starting point for future work with this instrument.

A. Multidimensional HPLC

Some of the fundamental questions which arise when designing multidimensional HPLC instrumentation include the following: What are the effects of the valves and tubing on extracolumn band broadening? What types of baseline disturbances are caused by column switching and how can these be minimized? How much imprecision can be expected when using totally time-based event control?

These types of questions must be answered before applications can be implemented and interpreted properly. The applications which follow are mostly taken from the fuel work which has dominated much of the instrument's use. Fuels are very complex samples, and therefore multidimensional chromatography is ideal for characterization and classification. This type of sample cannot be completely resolved, however, even with column switching techniques. Therefore, the samples shown do not produce chromatograms with the same degree of resolution as samples containing fewer components, such as those mentioned in the historical chapter of this dissertation. Nevertheless, it was felt that the most productive use of the instrument was to apply it immediately to the fuel analyses for which it was constructed. The chromatograms shown illustrate the use of the instrument and software.

1. Extra-column Band Broadening in MHPLC

In a multidimensional HPLC system a greater length of tubing is required than in standard HPLC because the outlet of each column must be returned to the valve to which the inlet is attached. One way of avoiding this problem would be to pack a column in a U-shaped tube. This is not as ludicrous as it sounds, since the shape of the column has no effect on the separation, and this design would allow the inlet and outlet of the column to be closer to the switching valve. In the absence of such a column, however, one can expect to use a minimum of about 2" plus the length of the column in connecting tubing for each switched column.

Figure 39 shows the setup used to make an extra-column band broadening comparison. In the first case, a mixture of toluene, anthracene and naphthalene were injected onto a 15 cm analytical silica column connected directly to a UV detector. The mixture was then injected again with two valves and lengths of tubing appropriate for connecting 25 cm columns placed between the silica column and the detector. The two valves were connected to the detector with a 1/16" low dead-volume union. The tubing used was 0.009" i.d. 1/16" stainless steel. The chart speed was 2.5" min^{-1}. The resulting chromatograms are shown in Figure 40. The average results for 6 runs are shown in Table 8.

An often-quoted measure of separation efficiency is the number of theoretical plates. In the absence of extra-column band broadening, the number of plates is fairly constant for different chromatographic bands. Because the number of plates calculated in Table 8 represents the separation efficiency of the entire system, including extra-column effects, the number of plates is not constant, but varies with retention time. Therefore, efficiencies for three reasonable (<10 min) retention times are shown. As

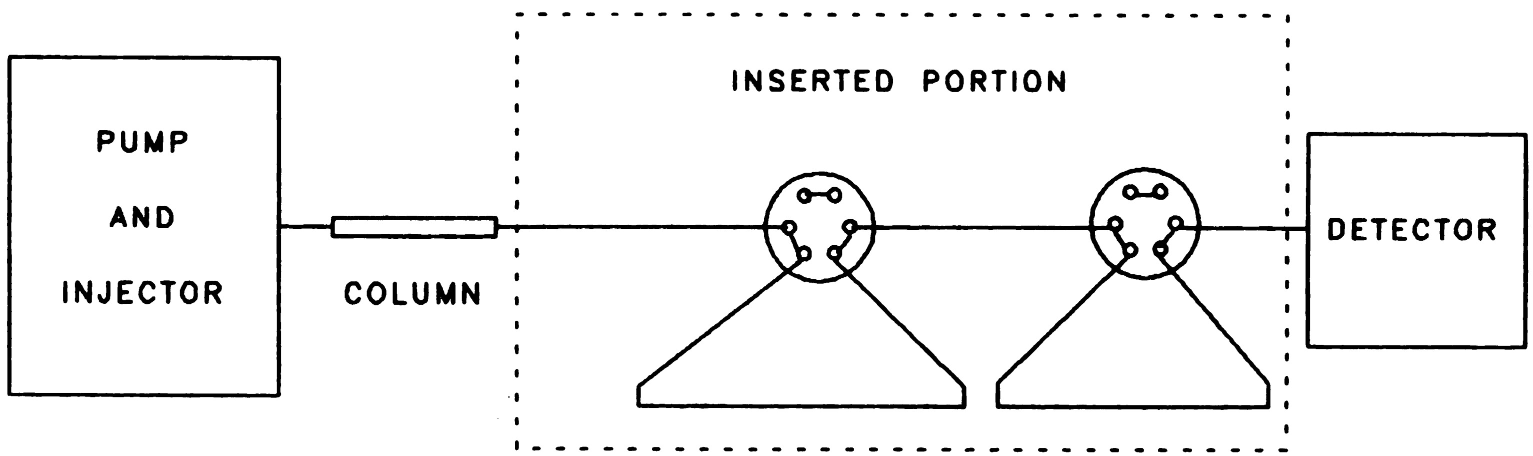


Figure 39. Configuration used for band broadening evaluation.

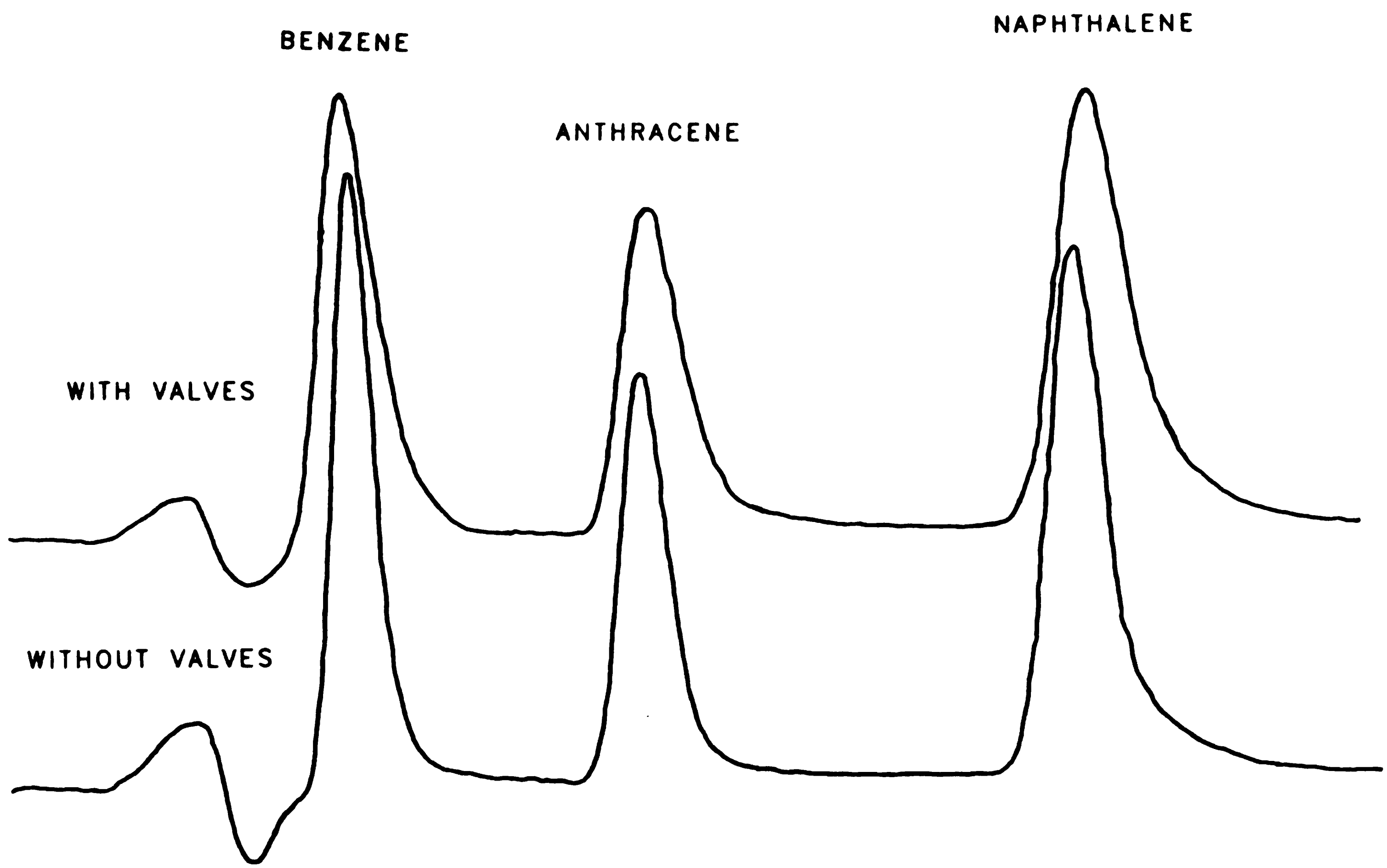


Figure 40. Representative chromatograms from band broadening evaluation.

Table 8. Peak Data for Extra-column Band Broadening Experiment.

Peak	Plates	Half-width (mm)	Retention Distance (mm)
1-No Valves	5145	8.7	266
2-No Valves	5785	9.9	318
3-No Valves	6361	11.7	396
1-2 Valves	3885	10.7	284
2-2 Valves	4543	11.8	337
3-2 Valves	5345	13.4	416
1-% Change	-24.5	+23.0	
2-% Change	-21.5	+19.2	
3-% Change	-16.0	+14.5	

Measured Resolution	Peaks 1-2	Peaks 2-3
Without Valves	3.17	4.15
With 2 Valves	2.67	3.64
% Change	-15.8	-12.3

expected, the added tubing affects the narrower, early-eluting peaks more than the later eluting peaks when stated as a percentage decrease in efficiency. As an alternative measure of band broadening effects, the resolution decrease for each pair of peaks is also shown.

As can be seen from these results, the relative effect of the band broadening depends upon the retention time of the peak in question. These results can be taken as the worst case using this size of tubing. In many applications, only a single valve is used, which would eliminate a lot of the extra tubing used in this study.

2. Baseline Disturbances in MHPLC

There are two sources of baseline disturbance in MHPLC. The first is a fairly symmetric departure from the baseline that occurrs immediately upon switching a column into or out of line. An example is shown in Figure 41. This disturbance is a result of the finite response time of the solvent delivery system to a change in backpressure when attempting to keep the flow rate constant. The flow cell used in the UV detector consists of a quartz plate pressed against a teflon spacer with flow channels cut into it. Because the reference side of the cell is not in-line, it does not respond to flow rate changes in the same manner as the sample side. The UV detector, which is slightly sensitive to the flow rate of the solvent through the sample cell, then detects the re-equilibration period. This disturbance is usually quite small and has a positive deviation for switching a column on-line (increase in backpressure, temporary decrease in flow). Switching the same column out of line gives a negative deviation of similar size.

The second type of deviation is only seen when a cut is made and developed with a different mobile phase composition. This type of deviation is a non-symmetrical, positive (higher absorbance) erratic disturbance due to the refractive index changes which occur as a result of mixing at the solvent boundaries. This disturbance can be

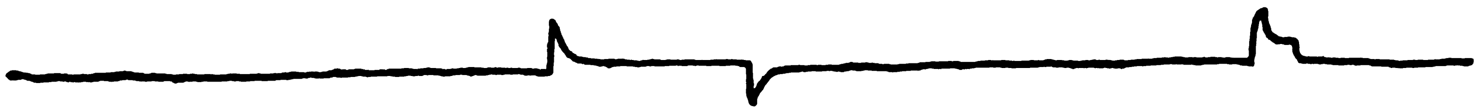


Figure 41. Example of baseline disturbance due to sudden backpressure change.

minimized by beginning development of the cut with a solvent mix similar to that contained in the cut portion and then performing a fast gradient to the desired solvent. If this is not possible, the size of the cut should be kept to a minimum. Figure 42 shows an experiment in which a plug of 100% THF was placed onto an ODS column and developed with 50% THF/H_2O. The size of the plug was varied from 0 seconds (the valve was immediately switched back, but a small quantity of solvent was cut), to 30 seconds. The program which automatically made these cuts is shown in Figure 43. A de-activated silica column was used as a backpressure source. Because this disturbance occurs at the void volume, it is seldom a major problem, but a blank should be run to distinguish this disturbance from actual peaks.

3. Effect of Imprecise Retention on Valve Switching

To be analytically useful, an MHPLC system must be able to perform a reproducible heartcut. This is quite difficult with time-based event control due to retention time imprecision. To measure this imprecision, an attempt was made to cut the center of a single chromatographic peak consistently to a second column. The critical valve switch would thus occur on the rising edge of the peak. The reproducibility of the height at which the switch occurs provides a measure of the reproducibility of the heartcut.

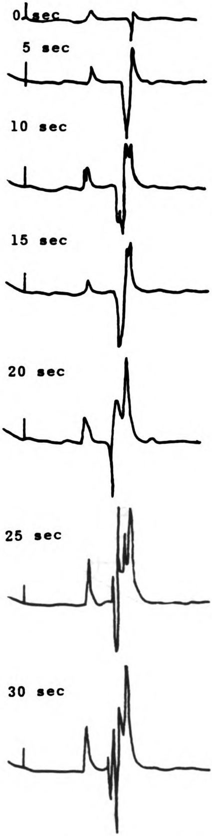


Figure 42. Examples of baseline disturbances due to refractive index change caused by mixing of heartcut solvent and developing solvent.

```
( BASELINE DISTURBANCE EXPERIMENT)
: THF %C ;    : H2O %A ;
: SILICA 2 CW ;    : ODS 2 CCW ;

: BDE DIGREC EDITMODE
  EDFI 1 ENTER# H2O 50 ENTER# THF 50 ENTER# FLOW 1 ENTER#
  EDFI 2 ENTER# THF 100 ENTER# FLOW 1 ENTER# RUNMODE ODS
  7 0 DO INIT 1 ENTER# FLOW? CLOCK 10 0 EVENT
  ODS CLOCK 10 0 EVENT
  SILICA INIT 2 ENTER# FLOW? CLOCK 10 0 EVENT ODS
  I 500 * WAIT I . ." SEC CUT" CR SILICA
  LOOP STP PRESS ;
```

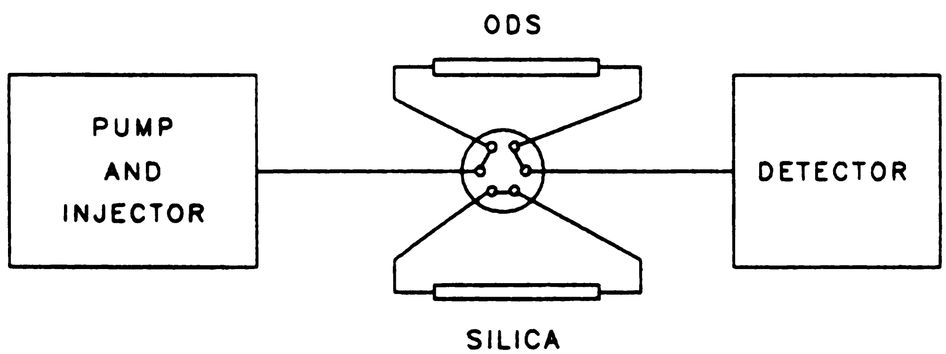


Figure 43. Top- Program used to automatically generate the previous series of baseline disturbance chromatograms. Bottom- Valving configuration used for baseline disturbance experiment.

The alternative method of performing the same heartcut is to specify a time window (or count peaks) and switch the valve when the detector output rises above a particular value. This method was accomplished using the command sequence:

INJECT 5 0 EVENT 20 %FSCHG 2 CCW

The command sequence for time-based switching was:

INJECT 5 48 EVENT 2 CCW

As can be seen from a comparison of the two command sequences, one disadvantage of time-based seqencing is that the exact time of the valve switch must be known. In both cases, the goal was to switch the valve at 30% above baseline (approx. 75 mm height). The results are contained in Table 9. The valve configuration used is contained in Figure 44.

Table 9. Heartcut precision comparison.

Run number	Time-based Cut Ht. (mm)	Detector-based Ht. (mm)
1	135.1	95.6
2	110.7	94.8
3	128.9	94.9
4	113.4	93.1
5	118.1	95.4
Mean	121.2	94.8
Std. Dev.	10.41	.986
% Rel. Std. Dev.	8.6%	1.0%

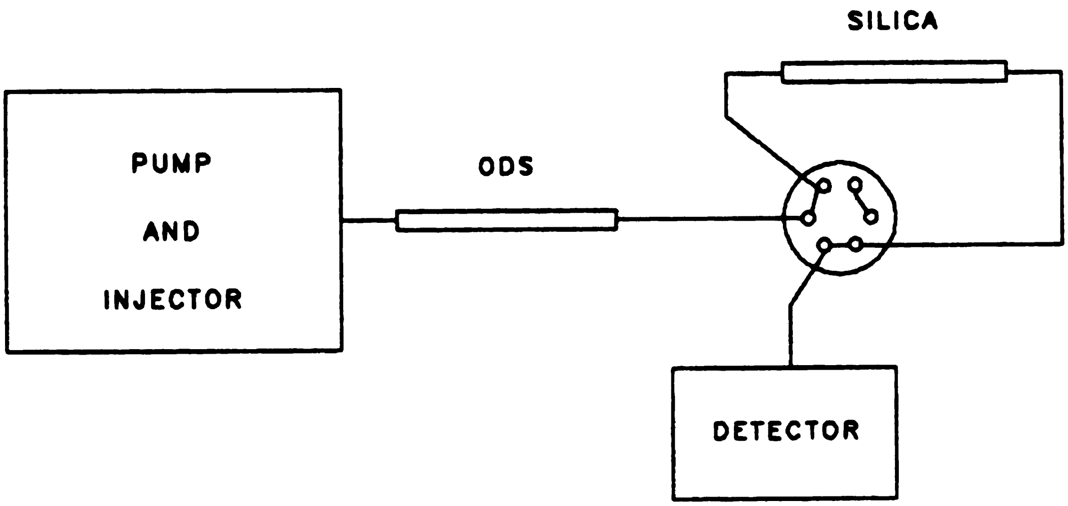


Figure 44. Valving configuration used to test detector-based valve switching.

As can be seen from these results, detector-based valve switching offers substantial improvements in the precision of peak cutting. The accuracy of the cut is more difficult to ascertain, however, due to the amount of sample trapped in the section of tubing between the valve and the detector. The amount trapped in the valve itself is negligible. Fortunately, the amount trapped in the tubing can be quantitated from a single run and the cut adjusted by subtracting the difference between the actual cut height and the desired cut height.

4. Backflushing Applications

One of the first applications of the system was a single-valve backflushing experiment. In this experiment, a 15 cm analytical silica column was connected as shown in Figure 45. With the valve positioned as shown, solvent flows in the forward direction through the column and on through the valve to the detector. With the valve in the switched position, the solvent flows in the reverse direction through the column, through the bypass loop, and again on to the detector.

A mixture of anthracene (1), nitrobenzene (2), and ethyl benzoate (3) was injected to give the first chromatogram shown in Figure 46. Without backflushing, peaks 1 and 2 elute in a reasonable time period, but peak 3 does not elute until nearly 44 minutes after injection. The

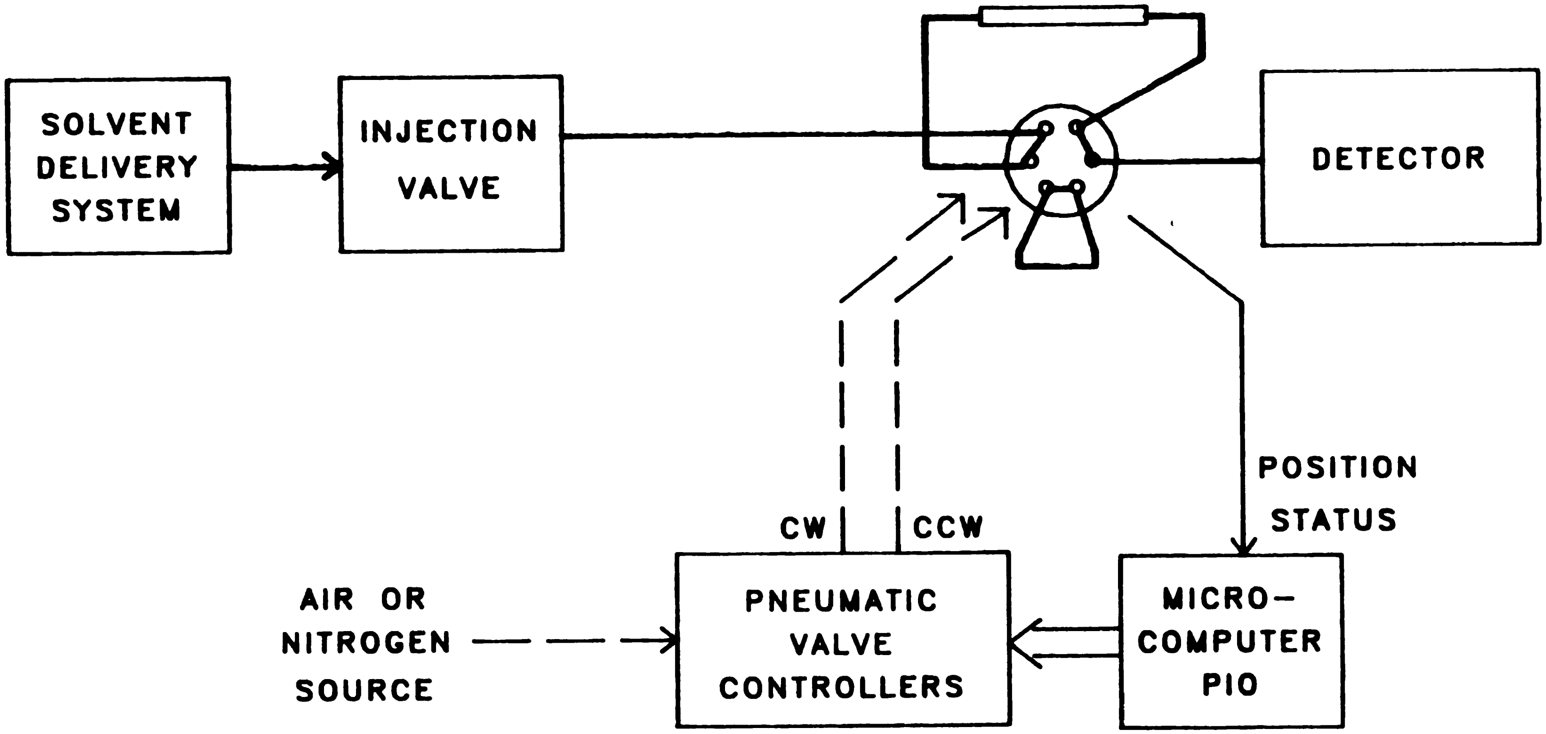


Figure 45. Single-valve backflush configuration.

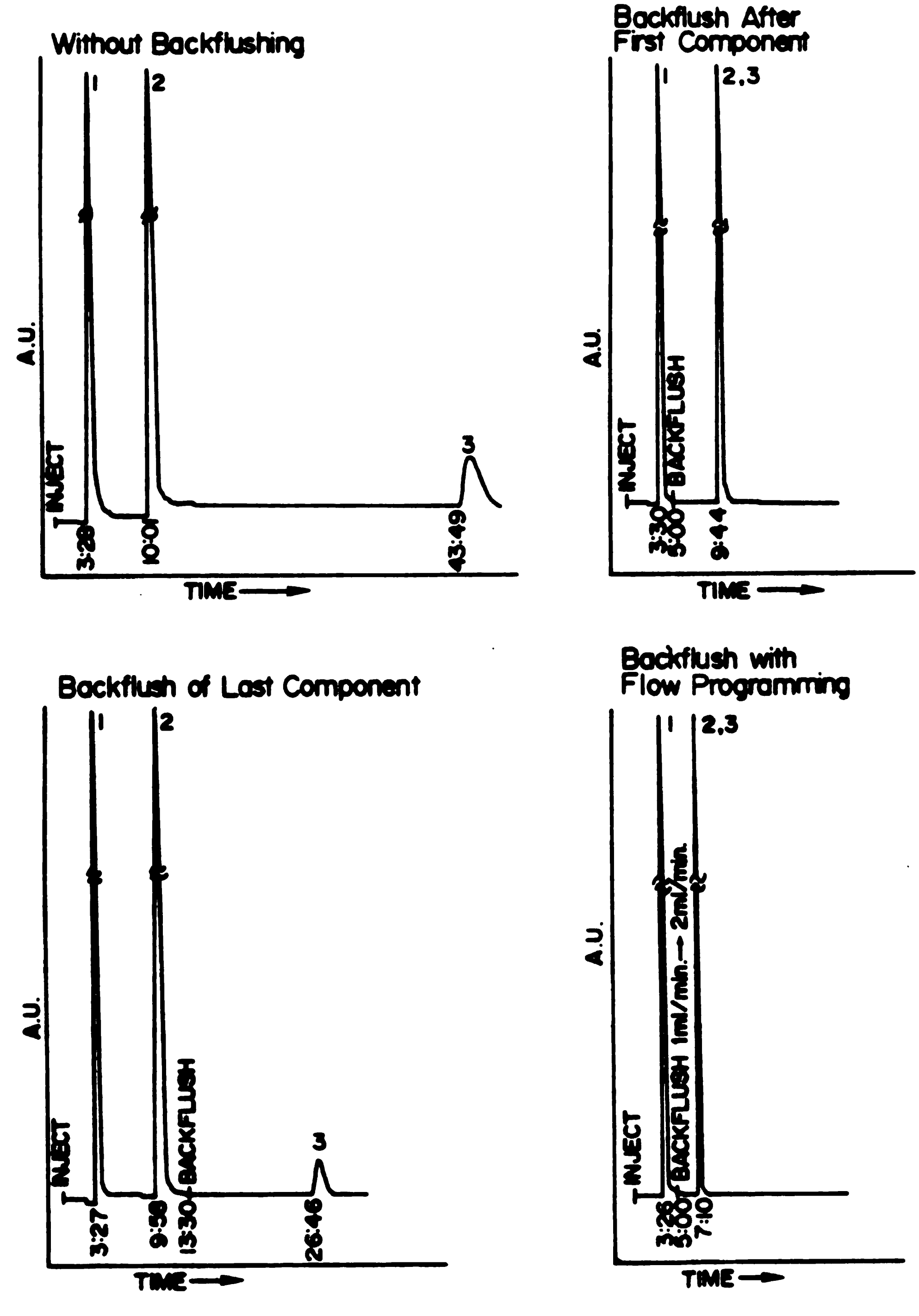


Figure 46. Examples of backflush chromatograms.

chromatogram in the lower left demonstrates how the situation is improved by incorporating a backflush after the second peak. The third peak now elutes at twice the backflush time, for a time savings of 17 minutes. This chromatogram also demonstrates one of the fundamental principles of the backflush. All components not irreversibly adsorbed to the column will elute at approximately twice the backflush time. In other words, it takes the peaks just as long to backflush off the column as it did for them to get into the column in the first place. This principle is often used in gasoline analysis to quantitate a group of components as a single peak, because all species will co-elute in a backflush.

In the third chromatogram a backflush is performed after the first peak, and the second and third peaks are co-eluting. Not only is an additional time savings realized, but the chromatographer can be sure that everything that is going to elute from the column has, in fact, come off after the 9:44 peak. This third chromatogram also demonstrates another point concerning backflushing. Very little time savings is realized for the second peak. This is due to the fact that the second peak had already gone about halfway through the column when the backflush was performed. Once a peak has gone more than halfway, it is faster to continue the normal elution process for that peak. An additional time savings can still be realized, however, if a synchronous flow increase is instituted along with the backflush as in the fourth chromatogram. With the help of the keypad emulator, a higher flow rate was employed to bring the total time down to only 7 minutes and 10 seconds.

Although backflushing is not as widely applicable as gradient elution, it can often achieve similar results with much less expensive equipment. The other advantages include minimal baseline drift and no need for a column re-equilibration period as in gradient operation. One of the nicest advantages of backflushing is that all compounds not irreversibly adsorbed will elute at a definite, pre-determined time. Thus, there is no danger of a strongly-adsorbed component eluting in the middle of a subsequent chromatogram.

5. One-valve Heartcut

The simplest heartcutting system employed was one using a single valve, as shown in Figure 47. With the valve in the position shown, a cut is being made from column 1 onto column 2. With the valve in the switched position, column 2 is isolated from the system and column 1 can finish being developed in the normal manner. When all components have eluted from column 1, column 2 is switched back in-line and development of the previously stored cut is performed.

Figure 48 shows a chromatogram obtained with this valving arrangement. The top chromatogram was developed on

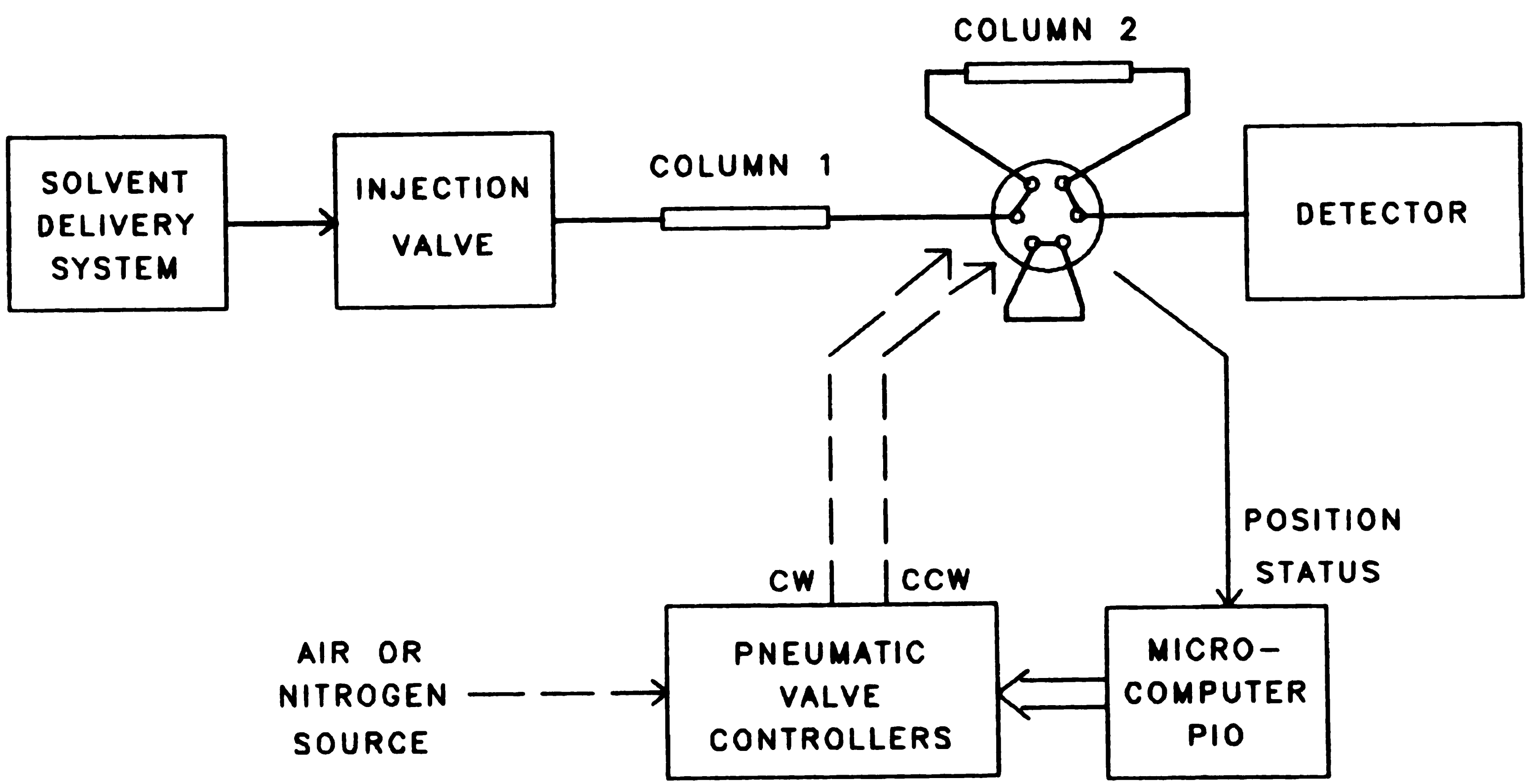


Figure 47. Single-valve heartcut configuration.

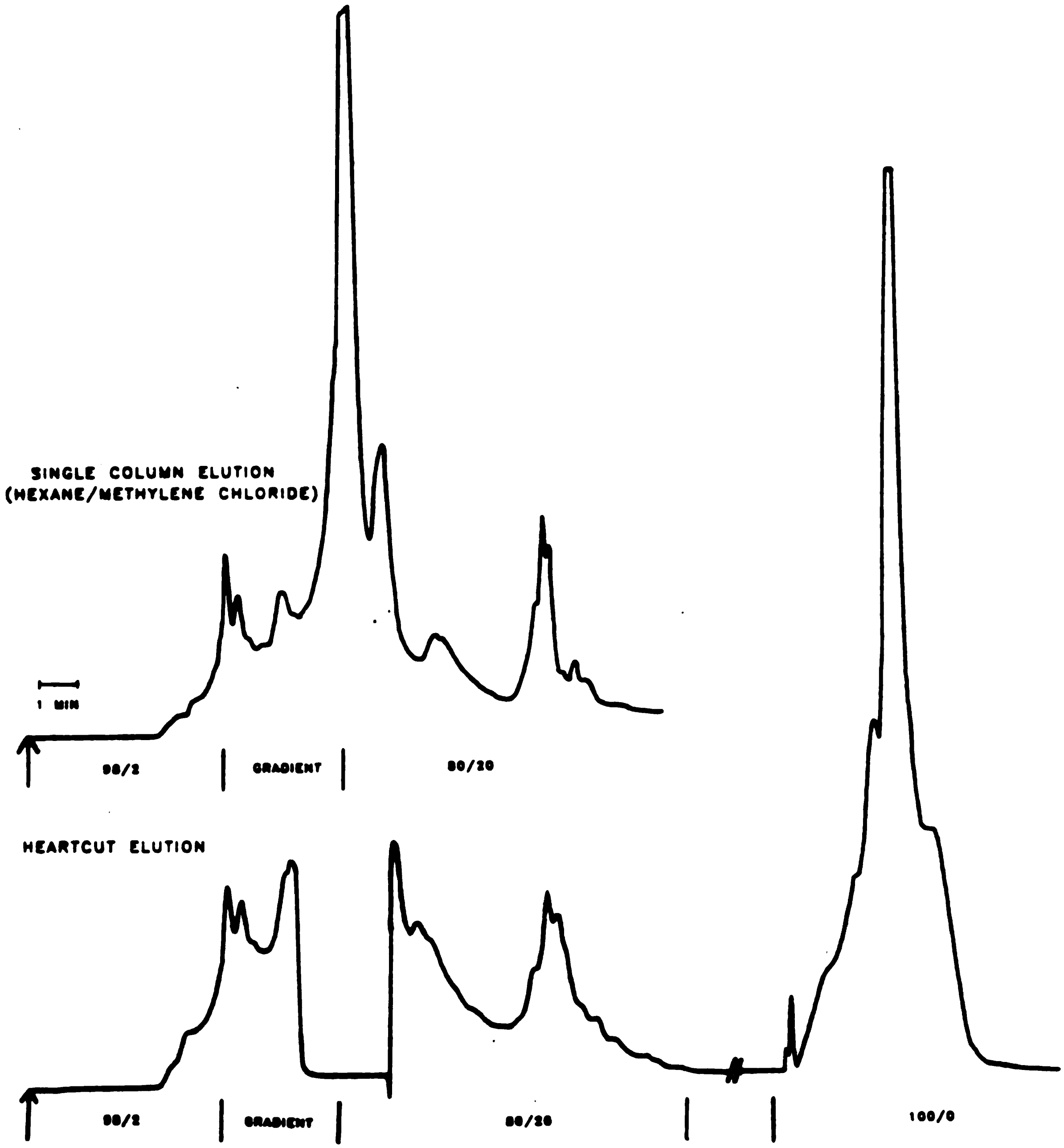


Figure 48. Single valve heartcut of residual fuel sample, amino column to silica column.

a 25 cm amino bonded phase column with the gradient shown. While this separation is quite good for a sample of this nature, it was desired to know if the largest peak was a single component or several unresolved peaks. To obtain some additional resolution, the peak was cut off the amino column (column 1) and placed on a 15 cm silica column (column 2) for further separation. After performing a solvent changeover to 100% hexane, the silica column was switched back on line for development. The resulting chrmoatogram shows evidence of several components being present in the heartcut.

The single-valve heartcut configuration has the disadvantage that the solvent used to develop the second column must flow through the first column. This configuration thus limits the choice of mobile phases to solvents which are compatible with both columns. Another disadvantage is that multiple heartcuts cannot be performed; the sample must be re-injected to perform a different heartcutting experiment. Fortunately, with a 2-valve system an autoinjector can be implemented along with the heartcut valve as shown in Figure 13. This can be used to perform an unattended multiple heartcut experiment.

The only other way multiple heartcuts could be performed with a single valve is shown in Figure 49. In this configuration, the first column is placed on the valve and switched into and out of line. The second column is

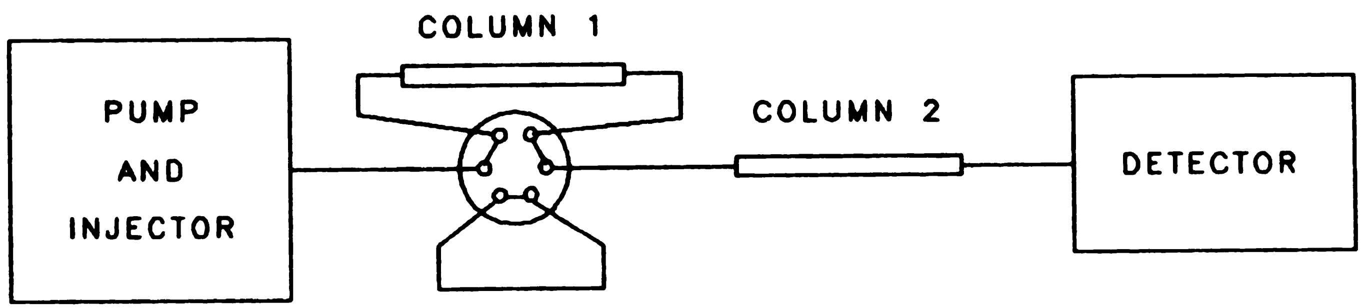


Figure 49. Sequential heartcut configuration using a single valve.

always in line after the valve. A heartcut is performed by switching the first column in line, letting some effluent be swept onto the second column, switching the first column out of line, developing the second column, and repeating until all components have eluted from the first column. This configuration is limited to sequential heartcuts of the entire first column.

6. Two-valve Heartcut

Two valves provide a much more flexible heartcutting arrangement. With the system configured as in Figure 50, either column can be isolated from the system. This allows the user to perform multiple heartcuts, but there isn't the limitation of having to perform sequential cuts of the entire first column. Another very practical advantage is the ability to develop the second column with a solvent that is incompatible with the first column. An example of this which has arisen in fuel characterization is the heartcutting of a portion of the effluent from an Ultrastyragel size exclusion column onto a reversed-phase column (see Danna (122) chapter 5). The Ultrastyragel column cannot withstand exposure to water, which was used as part of the reversed-phase solvent.

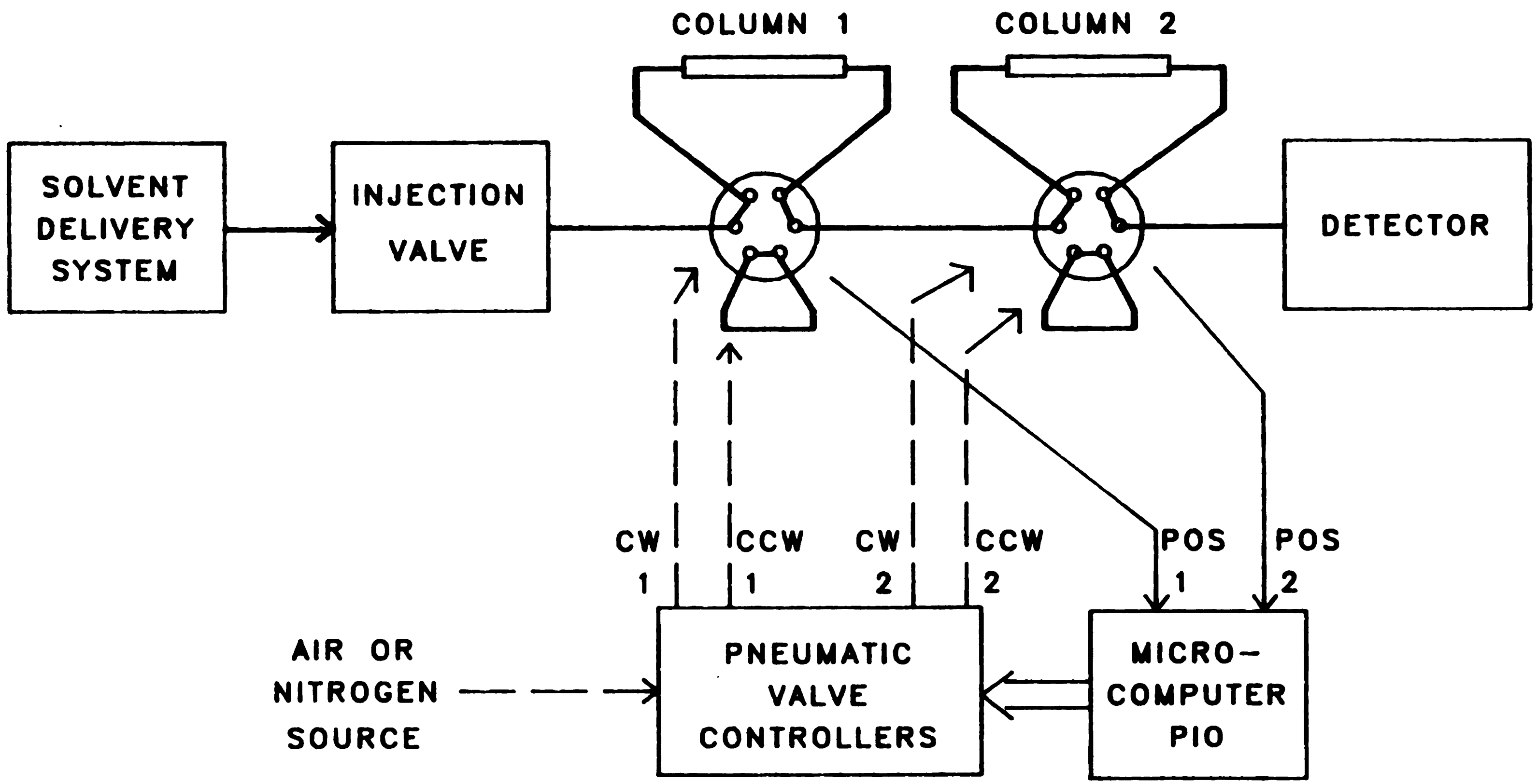


Figure 50. Two-valve heartcut configuration.

7. Multidimensional Backflush

A two-valve configuration which is especially appropriate for the separation of mixtures of widely differing polarities is the multidimensional backflush configuration shown in Figure 51. In this configuration column 1 is hooked up in the backflush mode, while column 2 is plumbed in the column isolation mode. Those components which are easily eluted are separated in the forward direction on column 1. The more polar species are backflushed off of column 1 onto the second column for separation with a different stationary phase.

Figure 52 shows a chromatogram which was developed with multidimensional backflushing. The top trace shows a four-component mixture eluted in the forward direction off of an amino bonded phase. The amino column can separate different ring numbers easily, as evidence by the resolution of the first three peaks, benzene, naphthalene, and anthracene. The fourth peak, however, contains a heteroatom which places its retention time close to that of anthracene. By backflushing the last two peaks onto a silica column and changing to 100% hexane, a much better separation of these two components is achieved.

8. Selectivity Programming with Backflush

A variation of the multidimensional backflush which uses three columns is shown in Figure 53. This

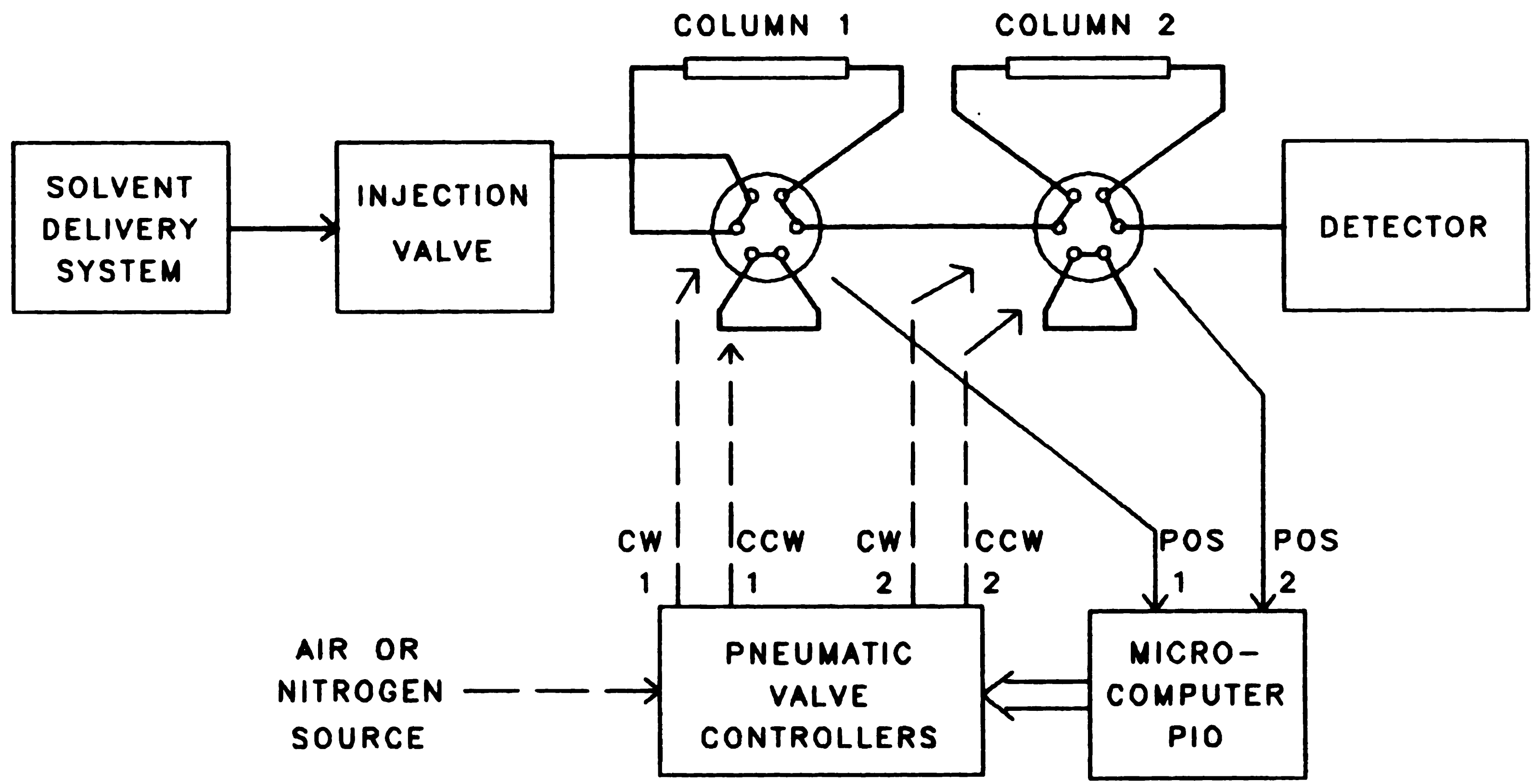


Figure 51. Configuration used for multidimensional backflushing.

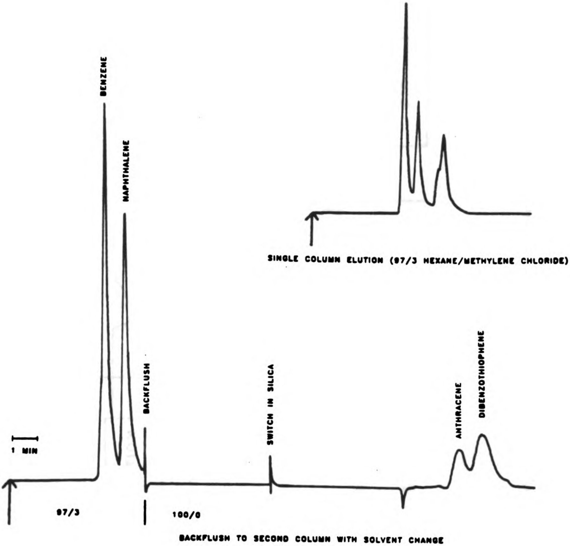


Figure 52. Multidimensional backflush of model compounds, amino column to silica column.

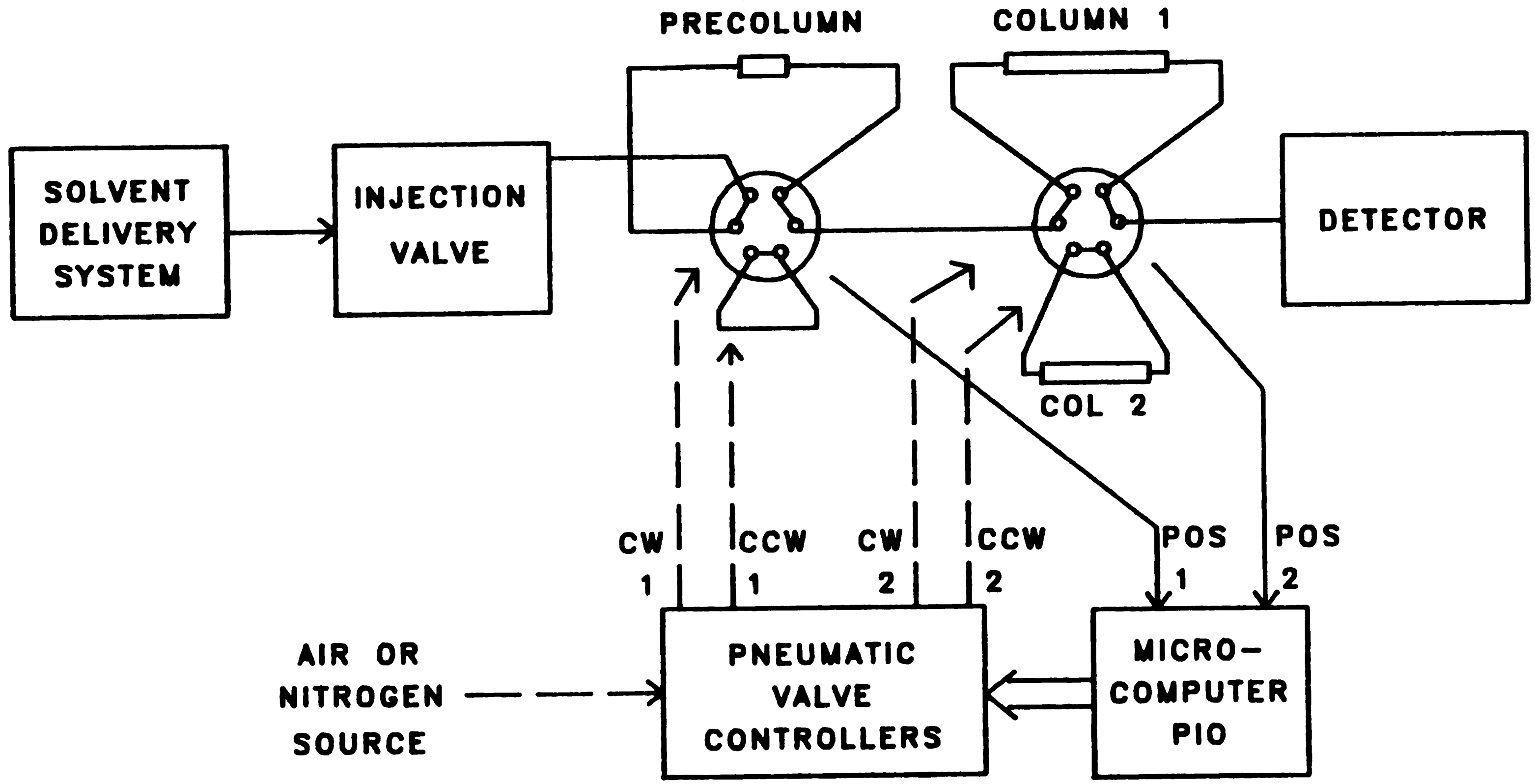


Figure 53. Configuration used for selectivity programming with backflush.

configuration uses a pre-column on the first valve in a backflush mode while the second valve is implemented in a column selection mode. The pre-column roughly separates strongly polar components from those easily eluted. The easily eluted compounds are allowed to pass to an analytical column for normal separation. The strongly retained components are backflushed off using a strong solvent and separated in a different mode on a different column.

Figure 54 shows a chromatogram obtained with this valving configuration. The pre-column was packed with an amino bonded phase, as was column 1. Column 2 is packed with an ODS reverse-phase packing. The sample is a portion of a residual fuel which had been previously separated off-line by silica low-pressure column chromatography. The fraction containing heteroaromatics was collected and injected onto the amino precolumn. The first large envelope is eluted in the normal phase with a gradient from 99% hexane/THF to 60% hexane/THF. Then a step gradient is performed to 100% THF with a couple of other components of medium polarity eluting. The remainder is backflushed onto the ODS column with a 30% acetonitrile/THF mobile phase and developed. Sharp peaks are obtained for the extremely polar moieties with this method, whereas they would have been extremely broad had they been developed solely in the normal phase environment.

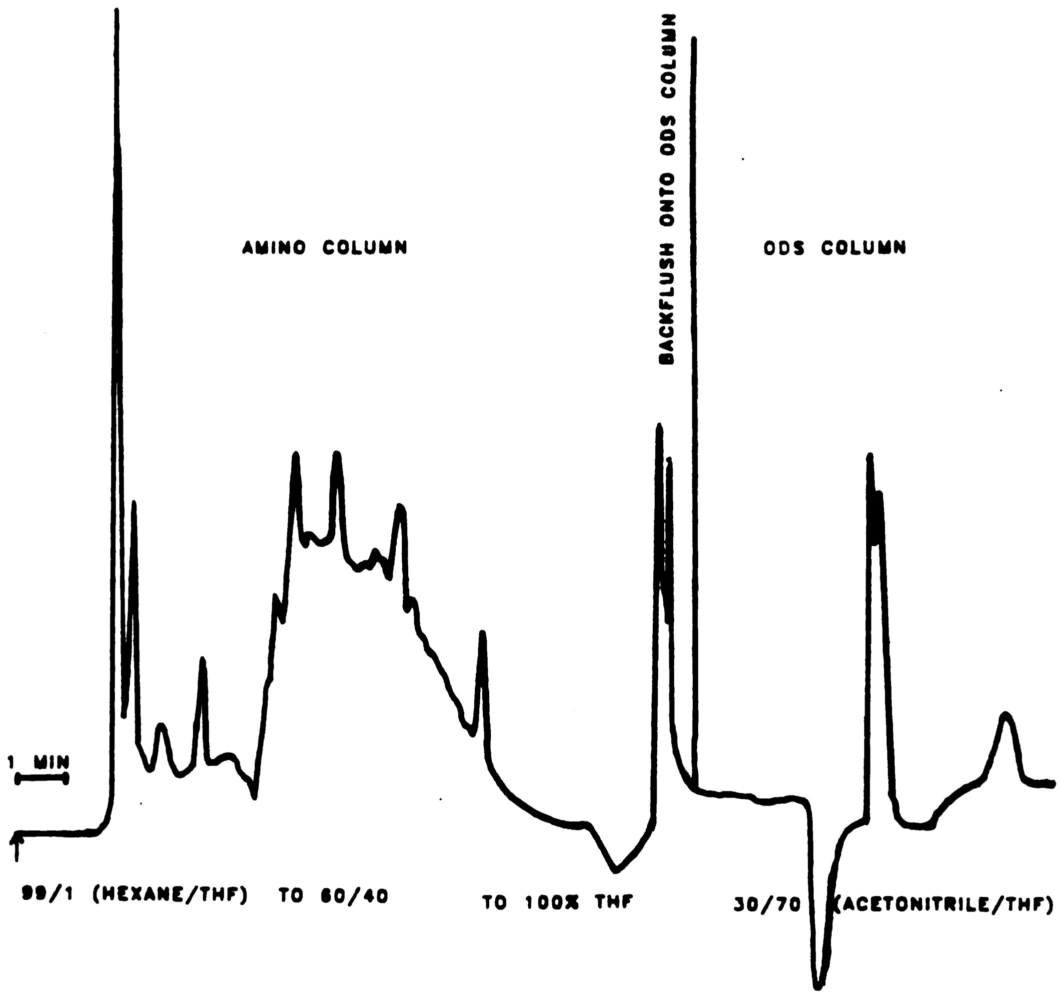


Figure 54. Selectivity programming with backflush on heteroaromatics fraction of residual fuel.

9. Autoinjector/Fraction Collector

Another interesting application of the system which has been implemented is the use of the system as an automated sample purification apparatus. By configuring the two valves as shown in Figure 55, a sample can be repeatedly injected, separated on a high resolution analytical column, and a particular fraction collected which contains the component of interest.

The autoinjector was implemented successfully, but the software had to be modified slightly. The words which turn the valve, CW and CCW, contain a software delay loop to allow the valve time to turn before checking to see if the valve has reached its new position. The addition of the sample vessel to the air line actuating the valve has a capacitive effect which causes the valve to turn more slowly. Therefore, two new words were written for use with the autoinjector which have longer delay loops. These words are SCCW and SCW and are loaded at the user's option from block 38.

10. Styragel Startup Routine

An excellent application of the SP8700 communication software is a routine which has been implemented to bring the Ultrastyragel column up to a flow of 1.0 mL min^{-1} gradually, according to the manufacturer's instructions. Because this column is packed with a gel, it is somewhat

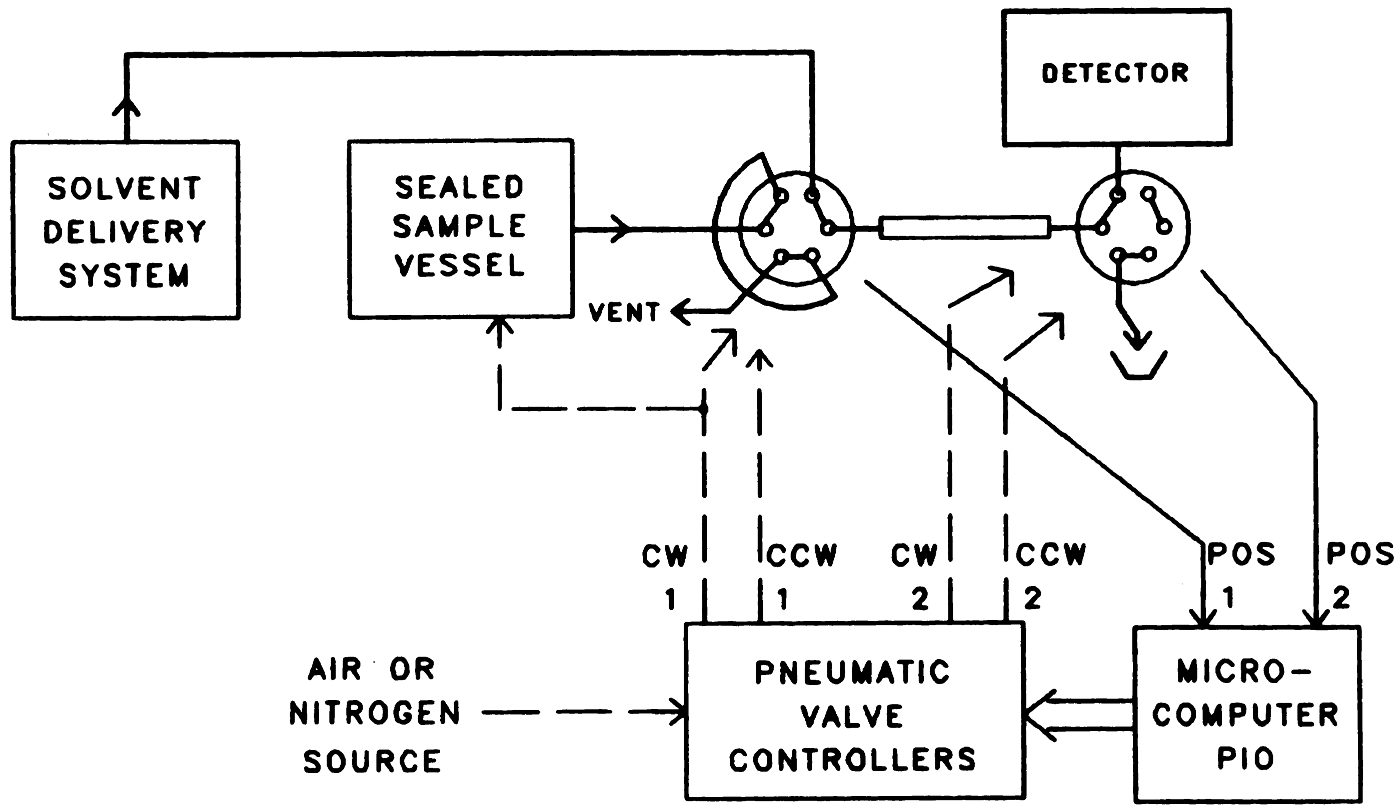


Figure 55. Two valves configured for autoinjection and fraction collection.

fragile and subject to bed compression when flow is increased too rapidly. The manufacturer recommends bringing the column up to flow in steps of 0.1 mL min^{-1} every 30 seconds. Unfortunately, this is very difficult to do on the SP8700 because the pump does not properly keep track of the flow rate unless at least one cam cycle is allowed per flow setting. At low flow rates, (e.g., 0.1 ml min^{-1}) one cam cycle takes several minutes. Therefore, a simple time-based flow increase is unsatisfactory. The styragel startup routine limits the flow rate increase at the low end by waiting for the cam marker, thus allowing the SP8700 to update the flow properly. The changes at the upper end are time-based, since several cam cycles occur in 30 seconds. In this manner the column is brought up to flow in the minimum amount of time with no operator intervention. This routine is loaded at the user's option from block 39.

B. Isolated Droplet Generator

In this section, the effects of various instrumental parameters on droplet generation are presented. Operation of the instrument in the stand-alone mode is described and compared with operation in the computer-controlled mode. The use of the pressurized liquid delivery vessel is then contrasted with HPLC as a liquid source. Specifically, the effect of a gradient on the droplet stream is examined in detail.

The primary application for which the droplet generator was constructed was for use as a liquid introduction system to a miniature nanosecond spark source. The results of this experiment are presented. Several other applications are also described.

1. Operation in the Stand-alone Mode

The instrument settings for droplet generation in the stand-alone mode are shown in Table 10.

Table 10. Initial settings for stand-alone droplet generation.

Source A: Internal
Source B: Internal
Bimorph Driver Source: Internal
Pulsing Trigger Source: Internal
Bimorph Driver Amplitude: 10.0 (Fully clockwise)
Pulsing Divider:1-5 drops/drop pulsed
Pulsing Voltage: 0 V
Frequency Adjust Toggle: Unlatched
Frequency Meter: Freqency, 0.1 sec
Frequency Meter Select: Bimorph Driver In
Deflection Voltage: 4 kV
Strobe Lamp: External Input, Low Intensity

The droplet generation procedure in the stand-alone mode is as follows. First, the liquid flow rate is adjusted to the minimum amount necessary to produce a jet of the desired velocity. The instrument is then turned on, and the bimorph driver frequency adjusted to the desired production rate. If the production frequency is not known, the frequency is slowly turned up from an initial setting of about 1kHz until a stable droplet stream is obtained. The frequency is then locked by placing the latch toggle in the "A/D Latched" position. Droplets are then pulled out of the main stream by increasing the pulsing voltage until deflection can be seen. The phasing is then adjusted by decreasing the bimorph driver amplitude slightly until only a single droplet is deflected, and the deflection is maximized. The bimorph driver amplitude should remain as high as possible for maximum stream stability. The number of drops/drop pulsed is then adjusted on the BCD thumbwheel.

Although this procedure is rather complicated, good results can be obtained with a little patience. Figure 56 shows the difference between the capillary stream with and without the applied bimorph oscillation. The top photo shows the stream with the instrument turned off and the irregularities and uneven size distribution is easily seen. The bottom photo shows the same stream with the instrument turned on and a bimorph oscillation frequency of 14.71 kHz applied. The droplets are uniformly spaced and monodisperse in size. In this case, the capillary size used was approximately 60 μm, and the flow rate was about 1 mL min^{-1}; this gave droplets of about 1 nL in volume or 64 μm in radius.

The frequency of 14.71 kHz is lower than predicted for the optimum droplet production frequency. Based on Rayleigh's equations presented in the instrumentation

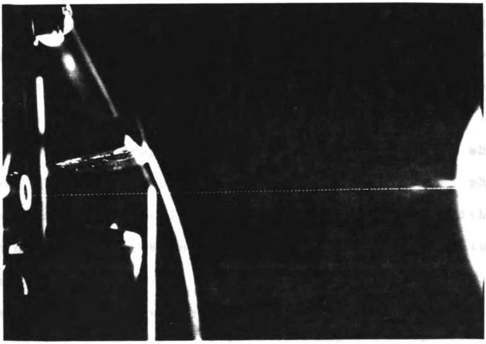


Figure 56. Top- Capillary jet without imposed oscillations. Bottom- Capillary jet with longitudinal oscillations imposed by bimorph.

chapter, the optimum droplet production frequency for a flow rate of 1 mL min^{-1} and a 60 μm capillary size should be about 25.1 kHz. In practice, it was found that imposing a square wave oscillation allowed much lower frequencies of droplet production than that predicted for a sine wave. It is believed that this is a result of the higher harmonics present in the square wave contributing to droplet formation. Upon close inspection, one can see that the stream first breaks up into pairs of small droplets which then quickly coalesce into large droplets produced at the fundamental frequency. This harmonic effect has the result of extending the lower range of droplet production frequency. For example, Table 11 lists some production frequencies obtained with a 30 μm capillary and a flow rate of 0.43 mL min^{-1}; these are well below the expected minimum frequency of 45 kHz. Although not all frequencies produce a stable stream, certain windows can be found which produce droplets at the fundamental frequency quite well.

Pulsing of droplets in the stand-alone mode is shown in Figure 57. The top photo shows the effect of poor phasing. More than one droplet is pulsed at a time and neither has good deflection. When the bimorph driver amplitude is adjusted as described above, the stream looks like the second photograph, where a single droplet is pulsed out of the stream. The droplet that is pulsed out in this manner is a charged droplet. Uncharged droplets can be produced as

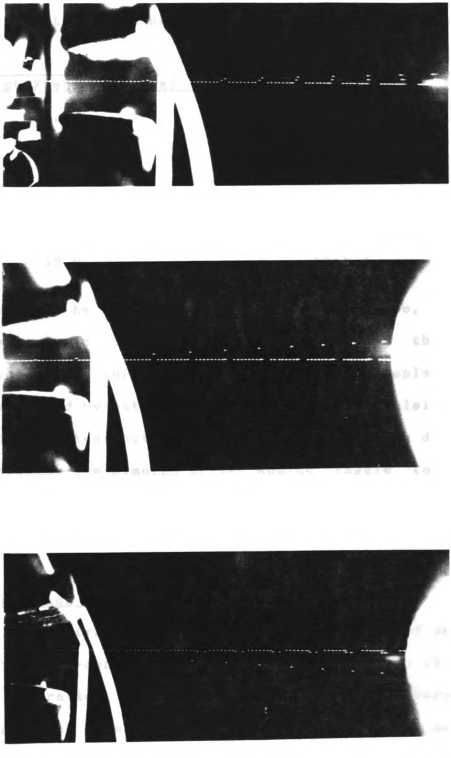


Figure 57. Top- Droplet stream showing poor phasing adjustment. Center- Droplet stream showing 1 charged droplet deflected. Bottom- Droplet stream showing 1 neutral drop separated.

Table 11. Low frequency droplet production characteristics.

Bimorph Frequency (kHz)	Stream Stability
1.0	Unstable
1.2	Unstable
3.2	Marginal
5.0	Stable
8.0	Stable
10.0	Stable
12.0	Stable
14.0	Unstable
16.0	Stable
18.0	Stable

shown in the third photograph. In this case, the rest of the stream is charged and deflected, and the charging electrode is turned off for a single droplet. This is accomplished by externally connecting the pulsing divider synchronization output to the external bimorph driver input and placing the bimorph driver source toggle to external. In either case, however, only a single droplet can be separated from the main stream in the stand-alone mode.

2. Operation in the Computer-controlled Mode

The instrumental settings for production of droplets in the computer-controlled mode are shown in Table 12.

Generation of droplets in the computer-controlled mode is much less painstaking than in the stand-alone mode. The frequency of droplet production is set using one of the two words IDG or RAMPIDG described previously in the software

Table 12. Initial settings for computer-controlled droplet generation.

Source A: Internal
Source B: Internal
Bimorph Driver Source: External
Pulsing Trigger Source: External
Bimorph Driver Amplitude: 10.0 (Fully clockwise)
Pulsing Divider:Not used
Pulsing Voltage: 0 V
Frequency Adjust Toggle: Not used
Frequency Meter: Freqency, 0.1 sec
Frequency Meter Select: Bimorph Driver In
Deflection Voltage: 4 kV
Strobe Lamp: External Input, Low Intensity
External Connections:AM9513 counter 1 to Bimorph Driver In
AM9513 counter 2 to Pulse Trigger In

chapter of this dissertation. If the frequency is not correct, it is adjusted in the highest resolution jumps possible by depressing a key. There is no need to lock or unlock the frequency adjustment. Phasing is automatically adjusted to approximate what is needed at each frequency. It is further adjusted from the terminal or under software control. This phasing adjustment is independent of the bimorph amplitude. Therefore, the bimorph amplitude can be kept at the maximum.

Once the droplet production frequency is set, the pulsing voltage is turned up until a suitable deflection is obtained. The phasing adjust keys are then used to maximize the deflection and center the pulse on the drop. If more than one drop is being charged the pulse width keys are used in combination with the phasing keys to optimize the pulse width and center it on the droplet packet. Utilizing these two sets of keys, a wide variety of droplet streams can be obtained.

Figure 58 shows some of the droplet streams which can be created under computer control. The top photograph shows a square wave of droplets, with 10 drops charged and 10 uncharged. Uneven duty cycles of droplets can also be produced as in the second photograph, which shows 5 drops charged and 15 uncharged. In fact, any number of drops can be charged and any number left uncharged, within the limits of the 16-bit counters used to produce the waveform. The longest pulse width that can be produced under computer control is 65.5 msec.

In the third photograph, the ability to control the charging pulse precisely is demonstrated. This droplet stream contains three drops which have received a full charge and two outer drops which have received a partial charge. This was accomplished by purposely shortening the charging pulse with the duty cycle keys and then interactively shifting the phase until the charge was properly balanced on the two outer droplets.

3. Use of the Constant Pressure Reservoir for Solution Delivery

The constant pressure reservoir described in the instrumentation chapter was constructed to deliver solution

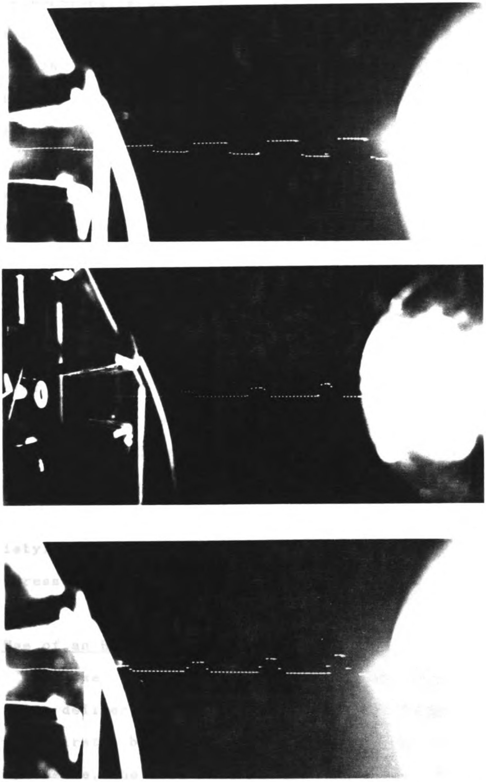


Figure 58. Top- Stream showing 10 charged and 10 neutral droplets. Center- Stream showing 5 charged and 15 neutral droplets. Bottom- Stream showing triangular droplet packet.

in non-chromatographic applications. A regulated nitrogen or air source at 10 to 70 psi is introduced through the cap. The bulk flow rate through the capillary is adjusted by increasing or decreasing the pressure. Flow rates were measured by collecting the effluent in a 10 mL graduate over a known interval. The relationship between flow rate and pressure is quite linear as shown in Figure 59. In practice, a calibration curve can be established for each capillary to aid in setting a particular flow rate.

One suggestion which may prove useful in maintaining the reservoir is to add a preservative to aqueous solutions to inhibit bacterial growth. The in-line filter was found to clog rapidly if old solutions were used. Of course, all solutions should be filtered prior to being put in the reservoir. A 0.45 μm nylon membrane filter of the type used in HPLC is recommended. Another solution to this problem may be to use disposable filters instead of the in-line variety. The filter should be able to withstand backpressures of 60 to 70 psi.

4. Use of an HPLC for Solution Delivery

The use of a reciprocating piston HPLC pump for solution delivery differs from the pressure reservoir in two ways. First, because the flow is constant regardless of backpressure, the flow rate is much easier to reproduce on a day-to-day basis. Unfortunately, most reciprocating HPLC

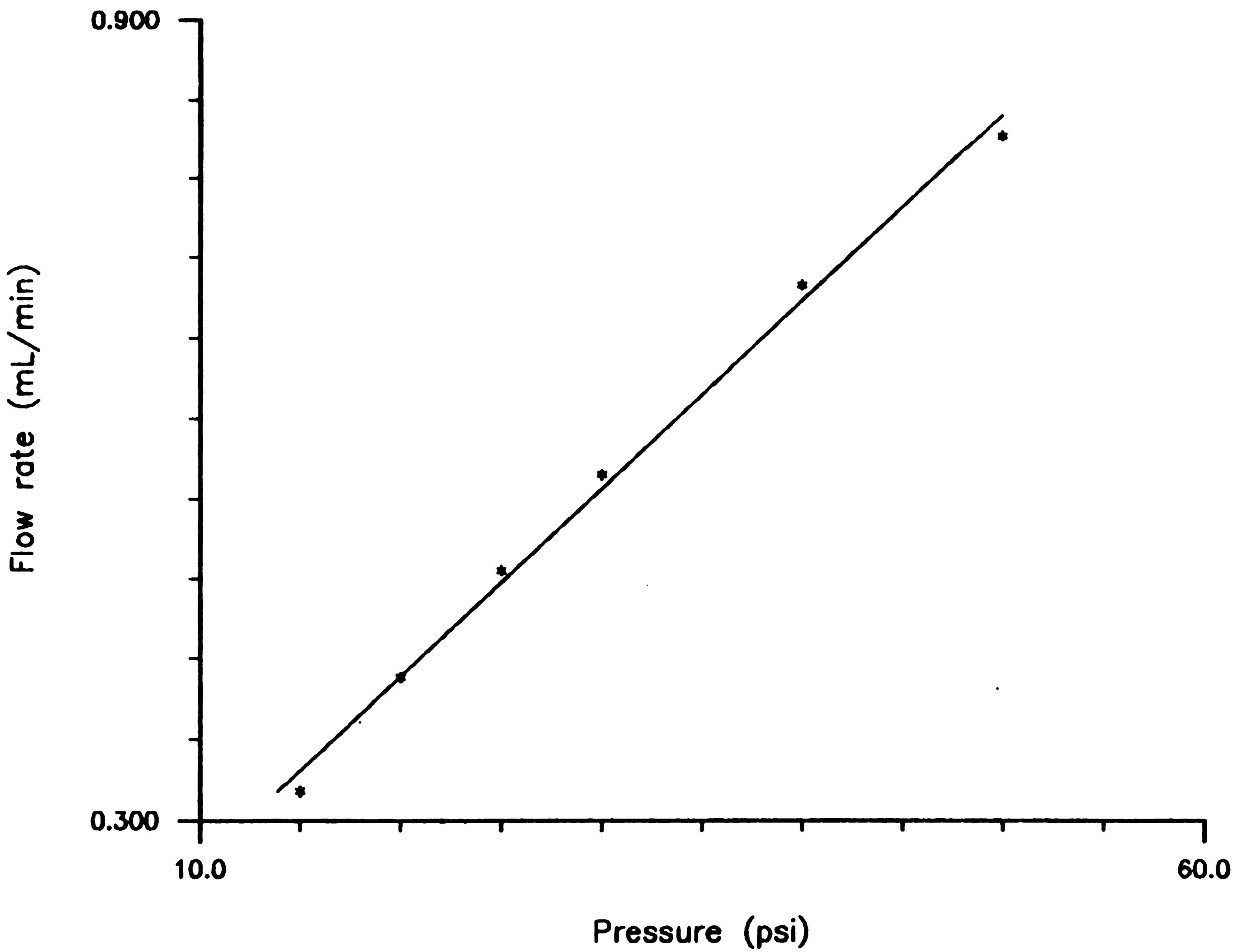


Figure 59. Flow rate/pressure relationship for a 30 micron diameter capillary and in-line filter.

pumps have some degree of pulsation associated with the flow due to the need to refill the piston chamber. The degree of pulsation is dependent upon the quality of the pump, the amount of backpressure the pump is pumping against, the compressibility of the liquid, and the ability of the system to dampen the pulses. The effect of the pulsations on droplet production was, of course, a major concern.

The SP8700 was set up to pump against a backpressure of approximately 1000 psi, and the aqueous effluent directed through the capillary. The droplet production parameters were set with the computer in the normal manner. For this system, the amount of pulsation was almost negligible, but a very slight visible movement of the drops along the direction of the jet trajectory was noticeable. If the droplets were charged in the normal manner, the result was a slight variation in the amount of charge delivered to each drop. Because this effect was so small, it was possible to compensate for it under computer control by lengthening the width of the charging pulse and using the phasing adjustment to center the pulse on the drop until no charging variation was observed. If the pulses were larger, as with a Milton-Roy single piston reciprocating pump, complete removal of the charging variation would be difficult, if not impossible. Fortunately, if neutral droplets are satisfactory for the experiment, all that is necessary is to impose enough charge on the droplets to deflect them into the trap. For this purpose, slight deviations in the deflection do not matter. The neutral droplets will follow a linear trajectory regardless of the pulsation.

5. Use of the Droplet Generator with Nonaqueous Solvents

Another important consideration for use of the droplet generator as an HPLC interface is the effect of nonaqueous solvents. All previous work on droplet making has been done with aqueous solutions. Three quantities important to droplet-making change when using different solvents. The first is the viscosity of the solvent. As the viscosity increases, one would expect droplet production to become more difficult due to increased damping of the applied oscillations. The second consideration is surface tension, which is one of the critical forces responsible for droplet formation. As surface tension decreases, droplet-making once again becomes more difficult. The third quantity is the ability of the solvent to conduct current. This is not an important consideration for droplet formation, but it could affect the charging of droplets.

To test the effect of changes in surface tension and viscosity, the SP8700 was programmed to deliver a binary gradient which goes through a large change in the above quantities. Methanol and water is one such system for which the surface tension and viscosity are known over a wide variety of compositions. The distance from the tip of the capillary to where the stream first coalesces into stable

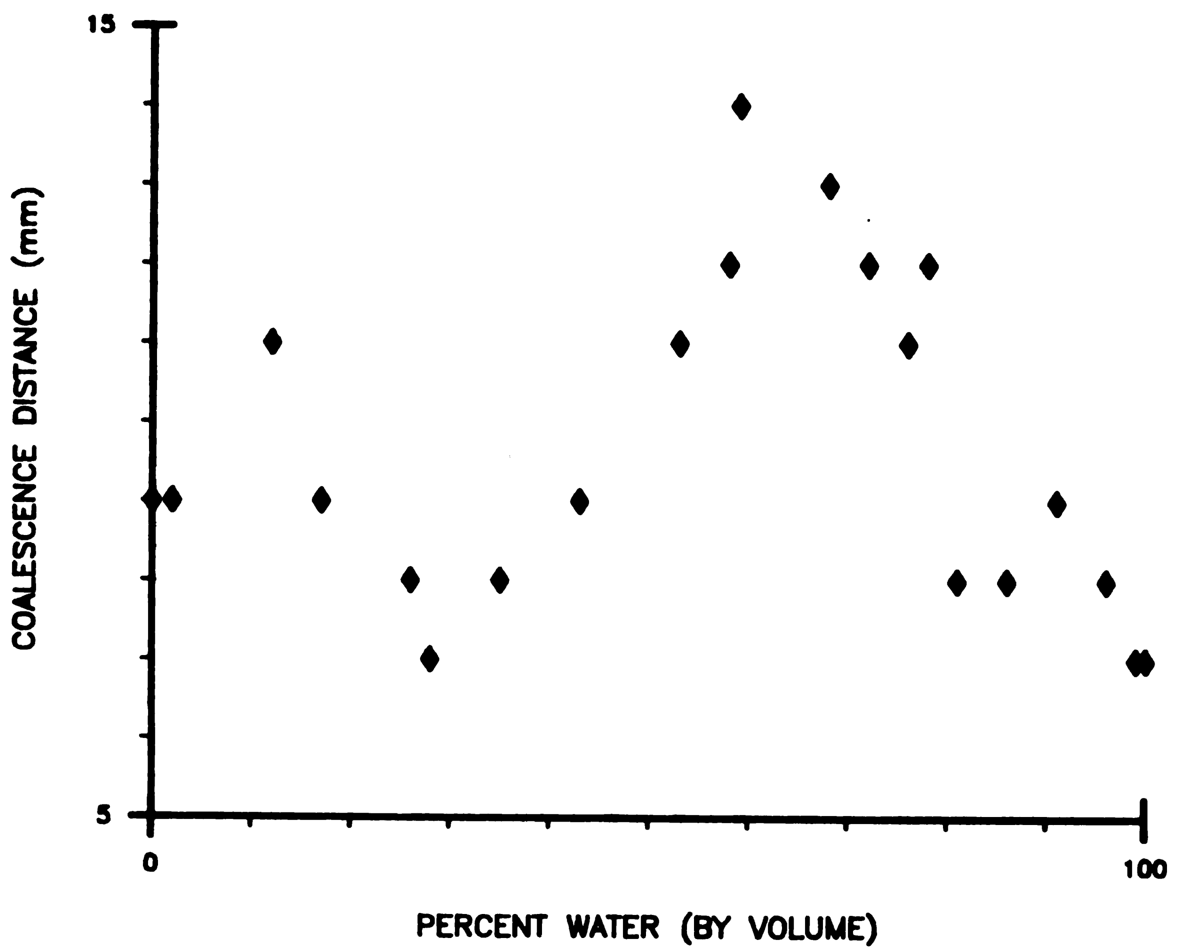


Figure 60. Effect of a 4%/minute water to methanol gradient on droplet formation.

droplets was noted at several points along the gradient. The results are plotted in Figure 60.

The first point to note concerning this graph is that the longest coalescence distance is only about 14 mm, which is well within the limits of the pulsing electrode. The other interesting feature is that there are two maxima instead of the one which was expected based solely on viscosity. The viscosity of the solution has been plotted on the same graph in Figure 61. The major trend is correlated with viscosity, but a deviation occurs at the 100% methanol end of the gradient. If the surface tension is plotted on the same graph as shown in Figure 62, one can see that this is the end of the gradient where the surface tension is very low. Since surface tension is a driving force for droplet formation, this may explain the second maxima observed near 90% methanol.

The effect of the varying coalescence distance on droplet charging is similar to that produced by improper phasing. The time interval for droplet coalescence is changing as well as the distance, with the result that a phase adjustment is necessary to keep the droplets properly charged. Using computer control, the necessary commands to adjust the phasing can be performed automatically. As in the case of pulsating flow rates, however, a better trajectory will be obtained by using the neutral droplet stream as opposed to the charged stream.

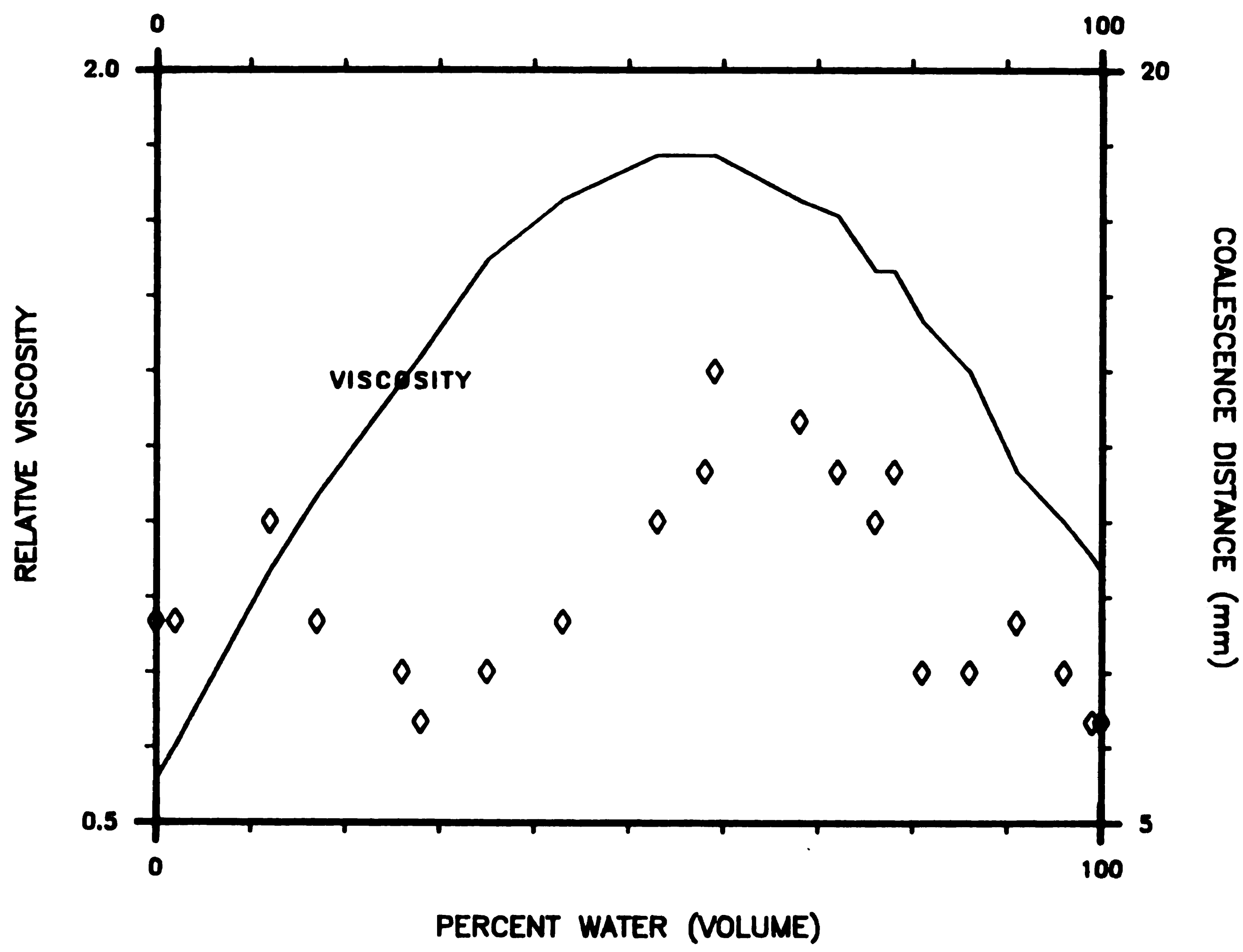


Figure 61. Effect of a 4%/minute water to methanol gradient on droplet formation showing viscosity change.

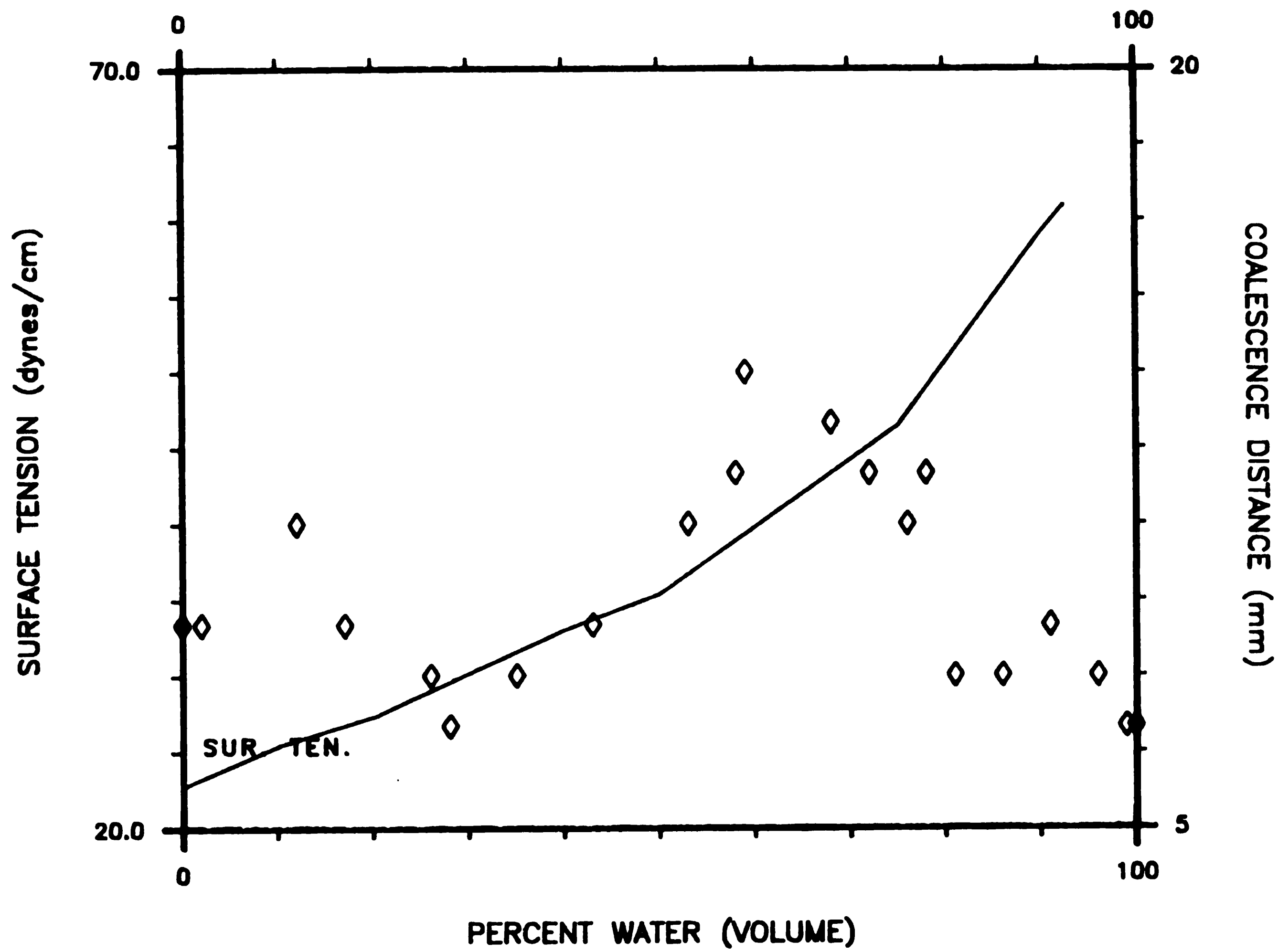


Figure 62. Effect of a 4%/minute water to methanol gradient on droplet formation showing surface tension change.

The ability to charge the solvent is dependent upon several factors. The primary factor is probably the conductance of the solution, since the only way for the current to be conducted to ground when a charge is imposed on the droplet is through the solution itself. The other factor which may be important is the dielectric constant of the solution, since this should influence the ability of the liquid to stabilize the charge separation necessary for a net charge to be imposed on the droplet when it separates from the jet. Table 13 shows some of the solvents which are suitable for charging droplets and some which were not able to be pulsed with charging voltages of less than 300 V.

Table 13. Solvent charging ability.

Solvent	Dielectric Constant	Specific Conductance (mho cm^{-1})	Chargable?
Water	78.3	6×10^{-8}	yes
Methanol	32.7	1.5×10^{-9}	yes
2-Propanol	19.9	6×10^{-8}	yes
Acetonitrile	37.5	1×10^{-7}	yes
Hexane	1.88	$< 10^{-16}$	no
CH_2Cl_2	8.93	4×10^{-11}	no
Tetrahydro-furan	7.58	4×10^{-10}	no

The addition of a small amount of a solvent with a high specific conductance can drastically alter the nature of the charging characteristics. For example, a 5% water/THF solution was able to be charged successfully.

6. Use of the IDG to Introduce Liquid to a Spark

One of the original applications of the IDG was to introduce liquid into the miniature nanosecond spark source (MNSS) shown in Figure 63. This spark source had been used successfully as a GC detector (123), but attempts to interface it to HPLC had been hampered by the lack of a suitable interface. The firing of the spark was controlled by a thyratron trigger module which could be operated externally by a TTL-based input waveform. The strategy for droplet introduction was to send the entire stream through the spark gap without pulsing out drops. The unused drops would simply exit through an opening in the opposite side. The firing of the spark could then be phased with the droplet production waveform so that a drop could be positioned exactly between the spark gap when the spark plasma forms.

To test this introduction technique, a 100 ppm calcium solution was delivered to the capillary using the pressurized sample reservoir. A 30 μm capillary was used at a flow rate of approximately .25 mL min^{-1} and an introduction frequency of 2.19 kHz. The droplet radii were approximately 50 μm. The spark was fired at the same rate, 2.19 kHz, so in this case all droplets were used within the

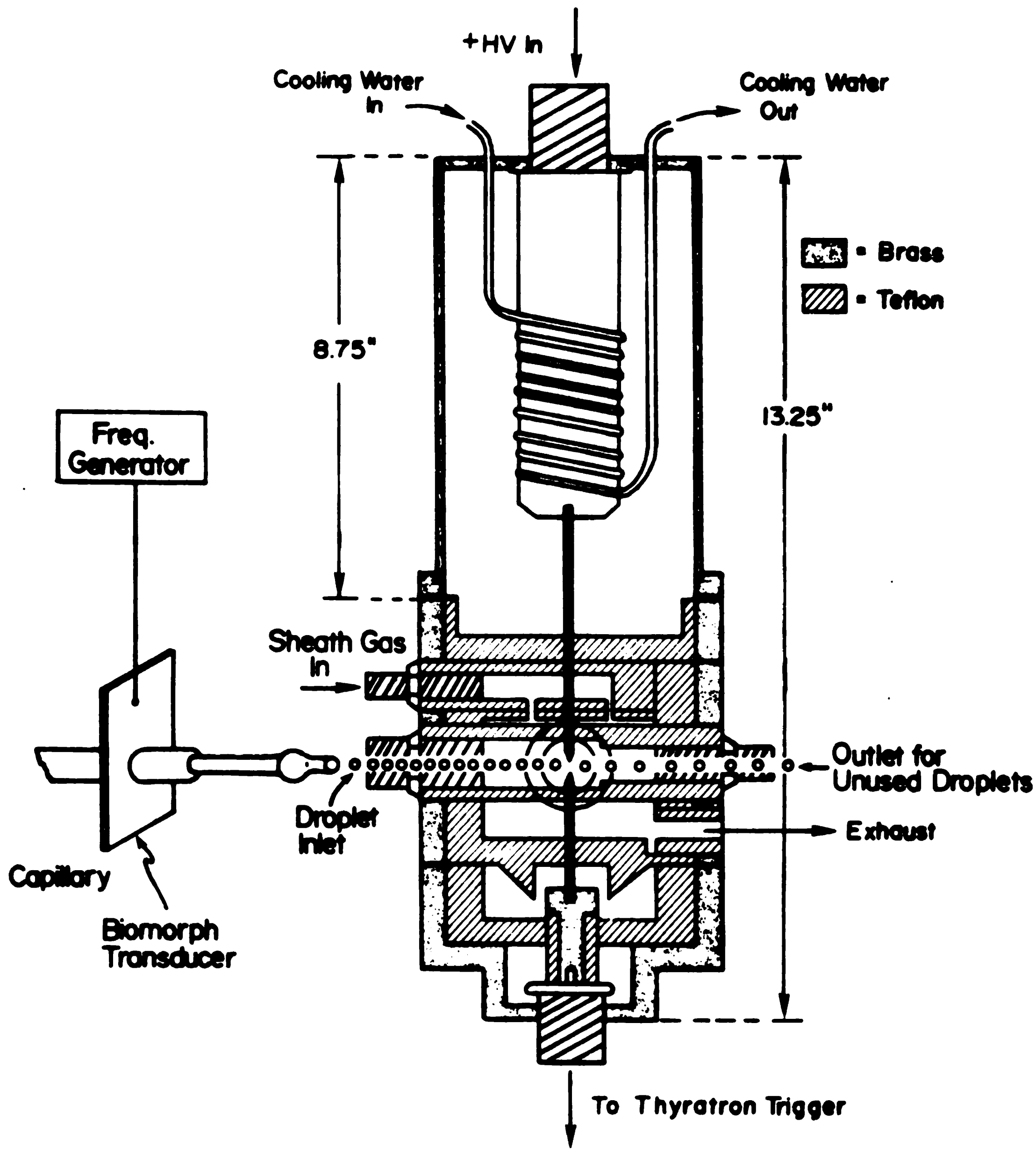


Figure 63. Droplet generator used with miniature nanosecond spark source.

spark chamber. Phasing was accomplished using the AM9513 timing controller as described previously.

The results of the phasing are illustrated in Figure 64. This part of the experiment does appear to be successful as noted by the minimum and maximum of the relative emmission intensity for calcium. The absolute magnitude of the signal, however, was very weak. Several different droplet introduction frequencies were tried, but none produced a strong enough signal to warrant use of this method in HPLC.

There are two possible explanations for this weak signal. The first is simply an intuitive feeling that the spark may not be of sufficient duration to completely desolvate and atomize the sample. While the spark itself is of very high energy, the duration of the spark is only a few nanoseconds. Therefore the total amount of energy dispersed may be too small for desolvation of droplets of this size.

The second explanation is based on a phenomenon observed visually in the spark gap when introducing droplets. Although great care was taken to line up the droplet stream with the spark gap, when the spark actually fired, remnants of the droplets were ejected from the sides of the spark plasma. Whether this is caused by sonic disruption or electrostatic repulsion, it is suspected that a large portion of the liquid did not remain in the vicinity of the spark plasma for a time sufficient for desolvation.

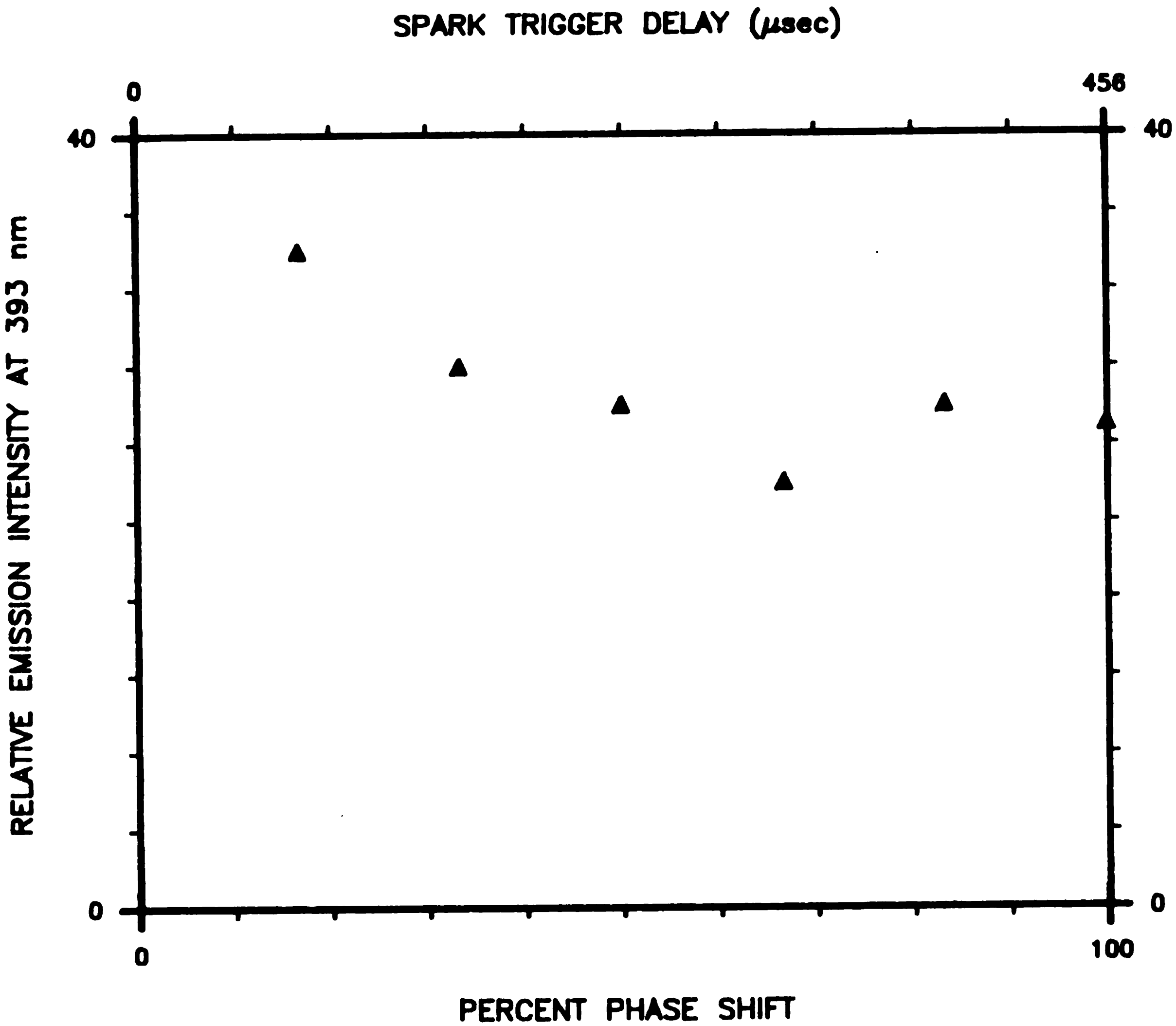


Figure 64. Effect of droplet introduction/spark trigger phase shift on emission signal.

7. Other HPLC Interfacing Techniques

A much more conventional excitation source for use with HPLC-AAS or HPLC-AES is a flame. The standard method of liquid introduction into a flame is the nebulizer. Several experiments were performed to assess the efficacy of this method of liquid introduction for HPLC interfacing.

The easiest way of interfacing the HPLC to the nebulizer is by direct connection. This method has the disadvantage of requiring the HPLC flow rate to approximately match the natural uptake rate of the nebulizer. Since most concentric nebulizers of the type used in AAS instrumentation have natural uptake rates on the order of 6-10 mL min^{-1}, a compromise must sometimes be made by raising the HPLC flow rate to a point above the optimum for the column. If the mismatch is too great, noise spikes may appear on the AAS signal. In certain cases, depending on the solvent used, a good match can be obtained. The chromatogram shown in Figure 65 demonstrates the results obtainable from direct connection using methanol as the solvent. In this case, normal HPLC flow rates were able to be used without the appearance of noise spikes.

A nickel diethyldithiocarbamate complex, $Ni(DTC)_2$, was synthesized in-house and checked for purity by HPLC-UV as shown in the top trace. It was not immediately apparent which peak was the complex and which was an impurity. By using nickel (232 nm) AAS detection immediately following

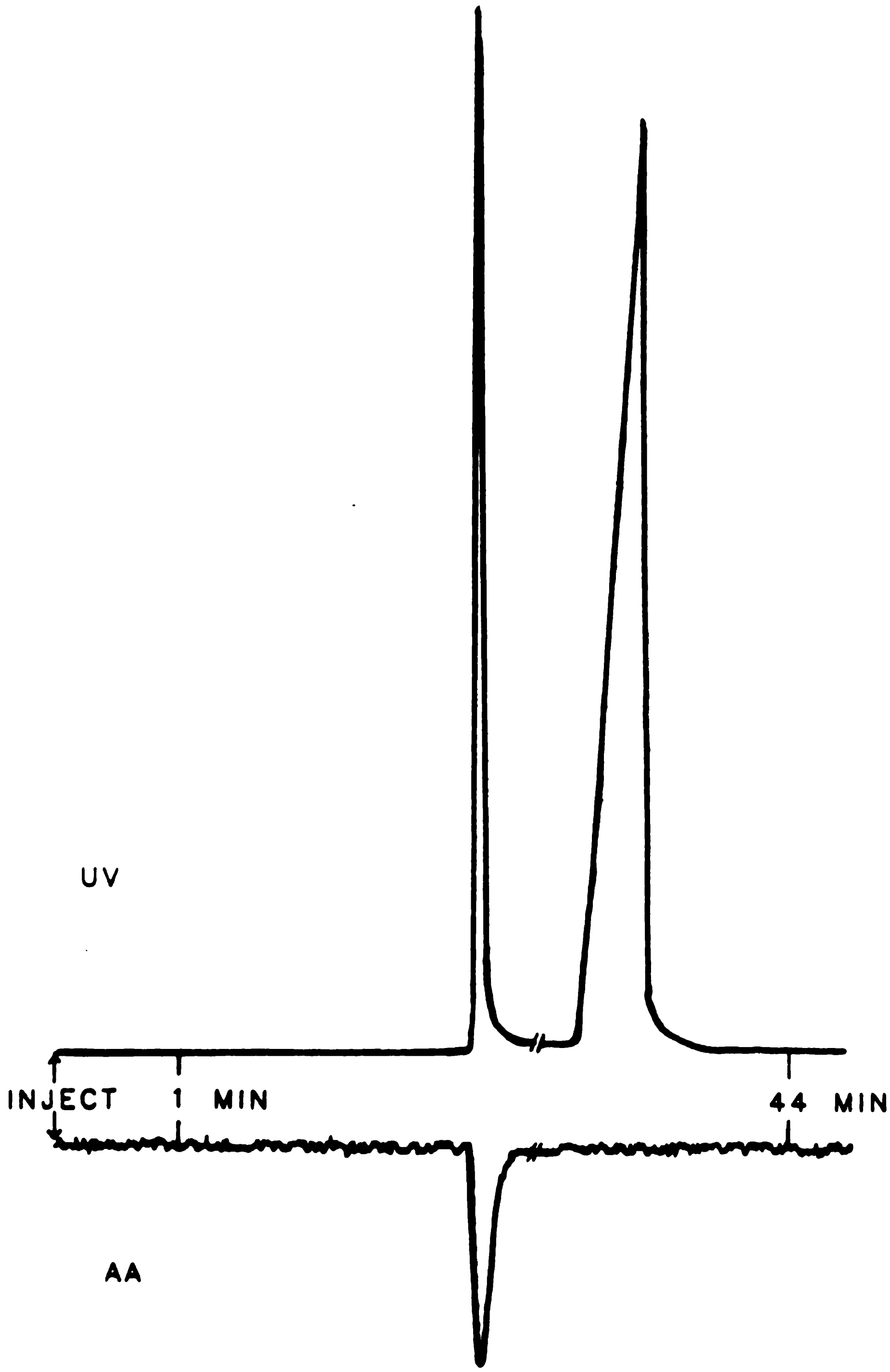


Figure 65. HPLC using AAS detection. Direct connect nebulizer interface.

the UV detector, the first peak was conclusively identified as a compound containing nickel.

Two other observations can be made concerning this chromatogram. First, it is apparent that the signal-to-noise ratio for AAS detection is considerably lower than for UV detection of this complex. While this trend is not valid for all compounds, for most metal complexes the signal obtainable from UV detection has a higher signal-to-noise ratio than that obtainable by AAS.

The second observation is that the width of the first peak has been increased considerably, even though a minimum amount of tubing was used to connect the UV detector to the nebulizer inlet. This band broadening is characteristic of nebulizer introduction and is dependent upon the exact nebulizer design used. A cross-flow nebulizer was also evaluated (122) and displayed much more broadening effects than the concentric nebulizer.

Another approach which has been employed with nebulizers is shown in Figure 66. The dripping cup interface allows the nebulizer to operate at its optimum flow rate independently of the HPLC optimum flow rate. Since the HPLC optimum for analytical columns is lower than the optimum for most nebulizers, each drop falling into the cup is immediately swept into the nebulizer and aspirated. This type of interface should increase the efficiency of nebulization by letting the nebulizer work in a "starved"

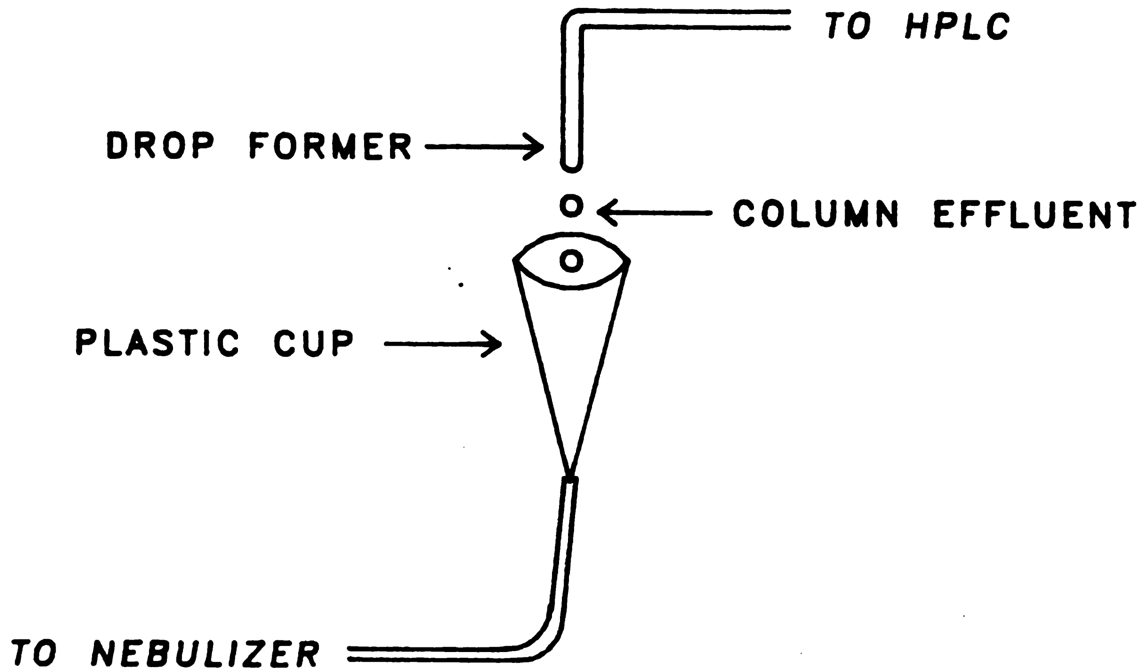


Figure 66. Dripping cup interface.

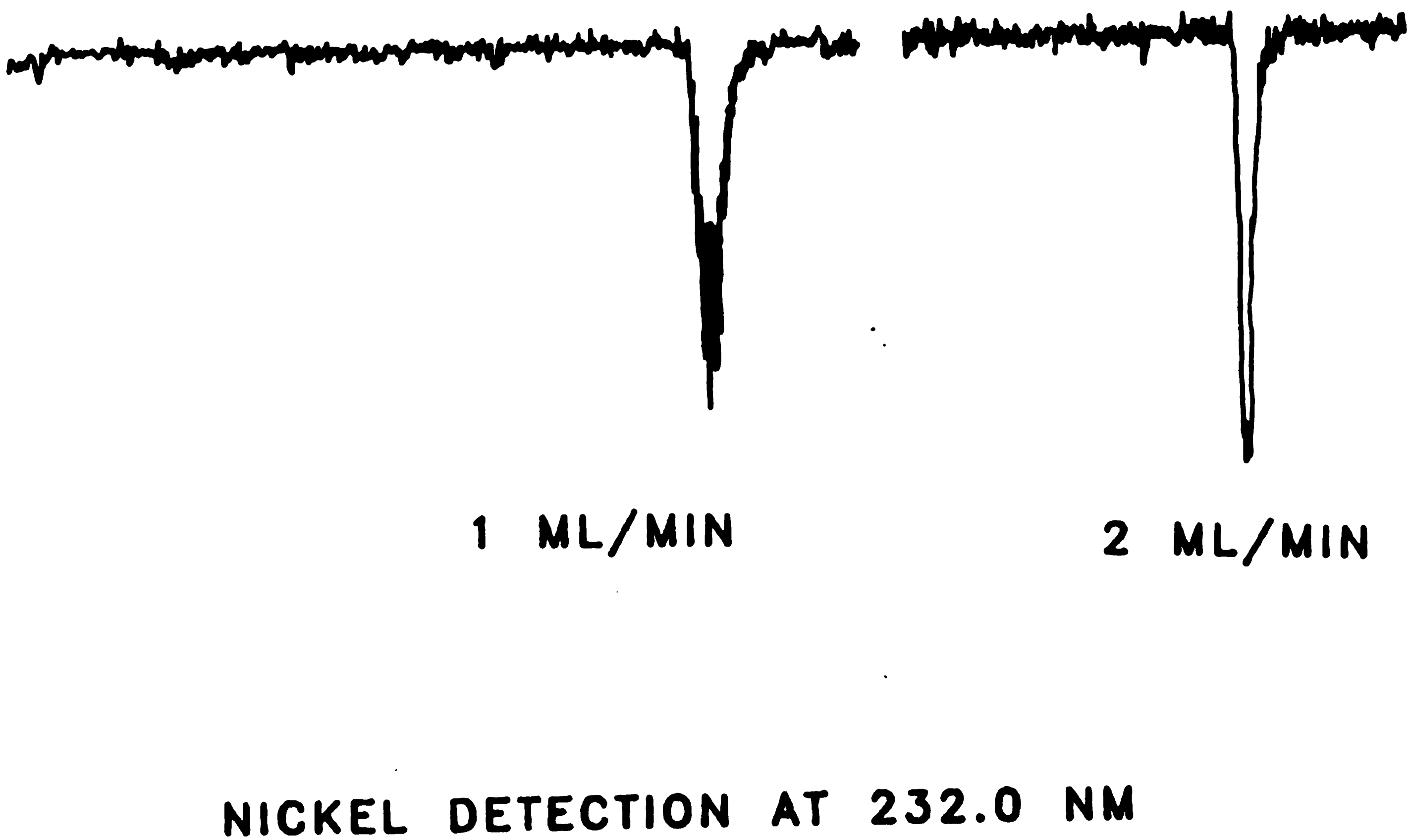


Figure 67. HPLC with AAS detection using the dripping cup interface.

mode, and also decrease the amount of band broadening by allowing the spray chamber to clear between droplets. The resulting chromatograms, shown in Figure 67, consist of a series of spikes, one for each droplet nebulized. The droplets should be large enough to give the same signal as in continuous introduction, but small enough to minimize mixing within each drop. Other researchers (77) have reported that for aqueous introduction, 0.1 mL droplets are sufficient in size to give the same signal as for continuous introduction. Unfortunately, for nonaqueous solvents, the surface tension is too low to form drops of this size.

Figures 68 and 69 compare results obtained with the direct approach with that obtained using the dripping cup method. Figure 68 compares the signal obtained by the two techniques for various flow rates. If the droplet size is large enough, the dripping cup method should be relatively insensitive to flow rate changes. The direct method is quite dependent on flow rate at low flow rates, but eventually levels off at high flow rates. The natural uptake rate of the nebulizer was about 2 mL min^{-1} for this solvent mix. In each case the best signal is obtained at higher flow rates.

Figure 69 shows the effect of different flow rates on the number of plates measured for the different techniques. The cup technique does appear to give higher plate counts, probably due to better clearing of the spray chamber between

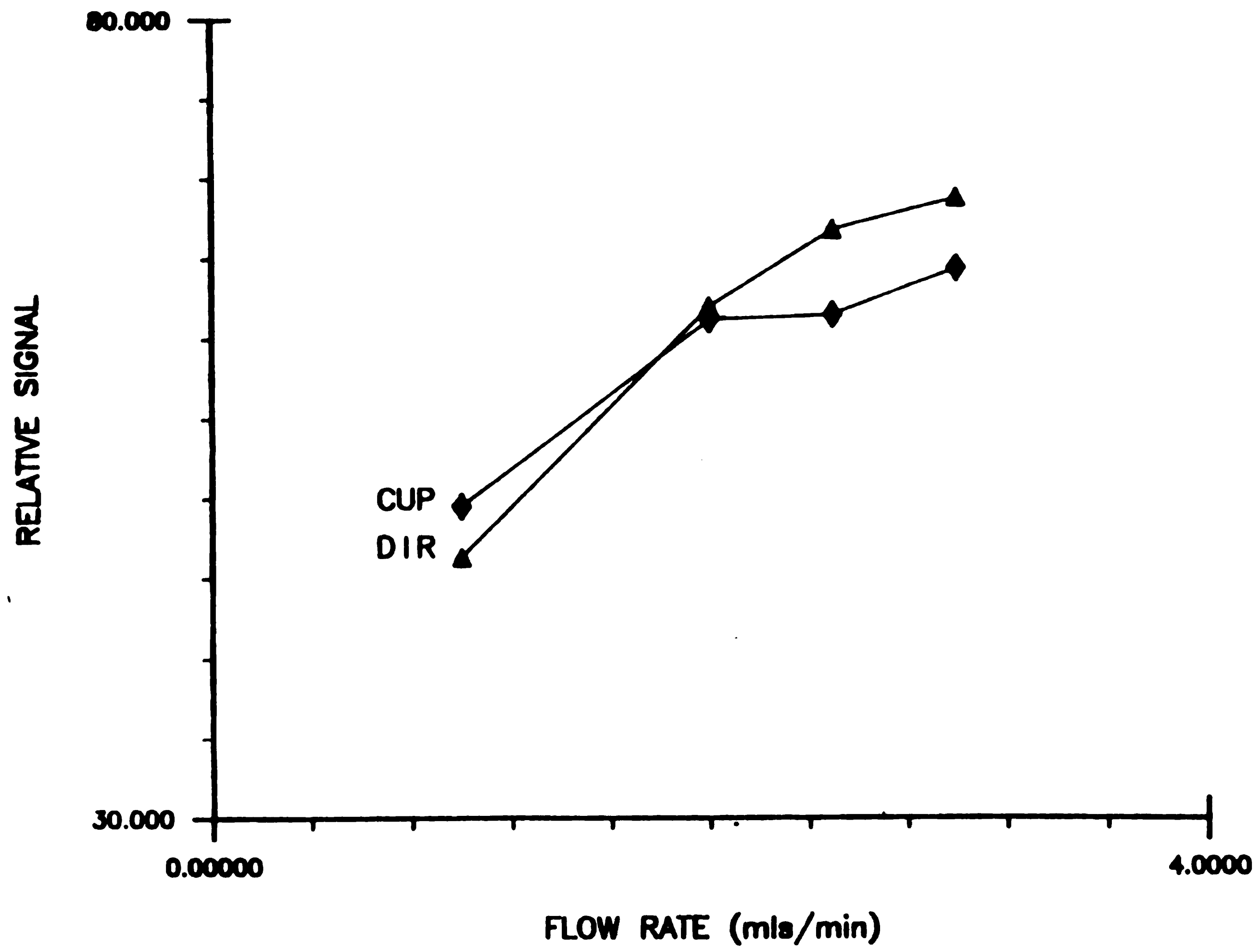


Figure 68. Comparison of signal magnitude for dripping cup and direct interface methods.

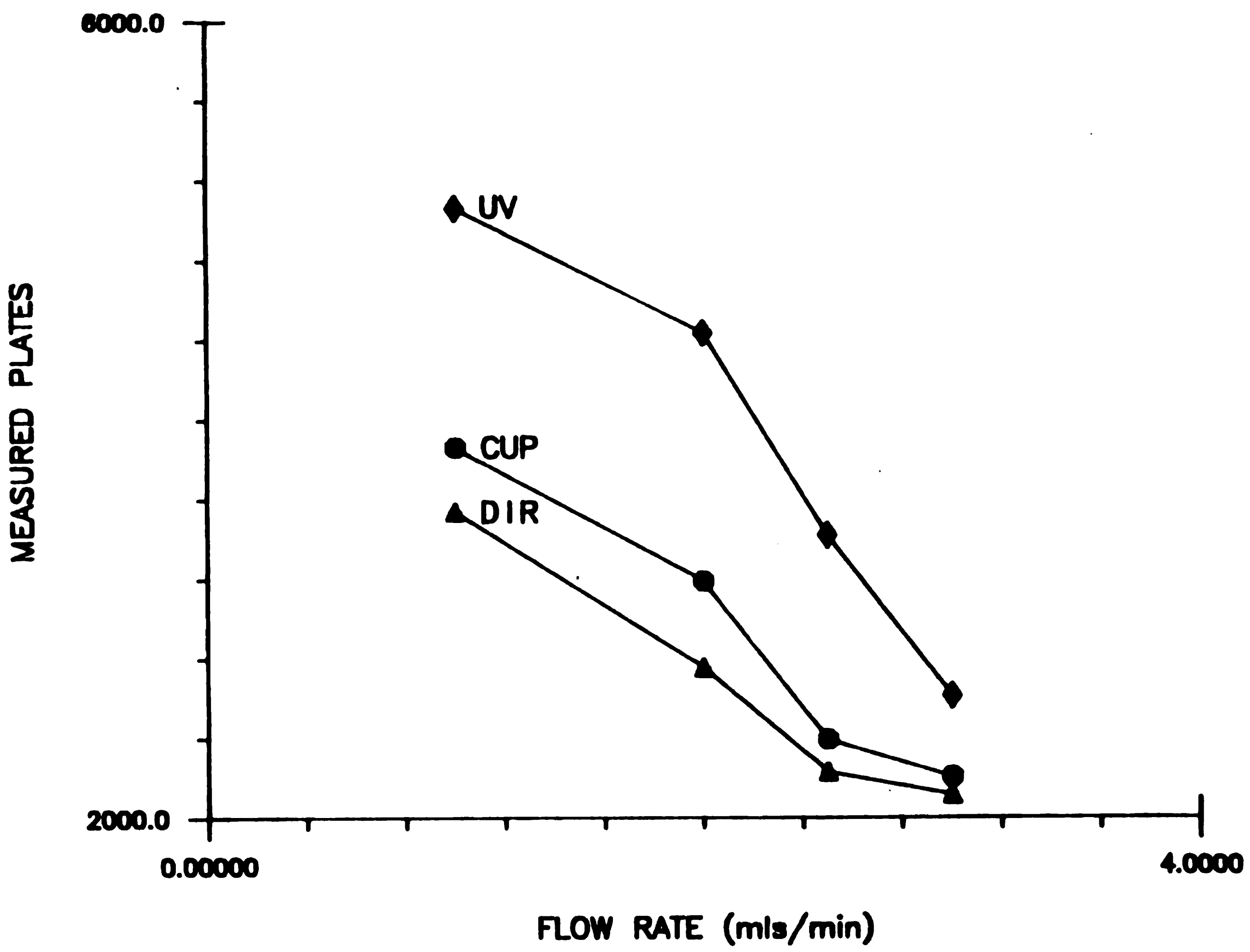


Figure 69. Comparison of band broadening for dripping cup and direct interface methods.

droplets. The chromatographic efficiency drops off considerably at higher flow rates due to operation of the column at flow rates above the optimum. This is evidenced by the plate counts measured from the UV detector signal.

The above results indicate that if signal magnitude is a primary consideration, the flow rate should be increased to a region where high sensitivity can be obtained. If chromatographic efficiency is the primary consideration, however, flow rate should be kept at a value which will maximize plate counts for the column being used. The inability to maximize both simultaneously is one of the fundamental problems of interfacing HPLC to element-specific detectors with nebulizers.

8. Use of the IDG with Flames

The isolated droplet generator has been used by several researchers as a method of introducing liquids into flames (99-101). The type of burner used has a large effect on the signal-to-noise ratio observed when using this method. Three types of burners were evaluated for use with the IDG. The first was a slot-type burner commonly used for AAS. Although this burner was able to desolvate, atomize and excite the sample, the emission occurred very high in the flame. In this region, the flame position is very susceptible to drafts. Thus, the portion of the flame viewed by the monochromator varied drastically, resulting in a large change in signal intensity.

The second type of burner used was a standard Meeker-type laboratory burner with an air-natural gas flame. This flame had the advantage of being taller than the slot burner and less susceptible to room drafts. However, it was also slightly cooler than the air-acetylene flame used in the slot burner. Nevertheless, this flame gave considerably better results. A picture of the droplet introduction into the flame is shown in Figure 70. These droplets are being produced at a rate of about 30 kHz in the stand-alone mode, with one out of every 900 droplets produced deflected into the flame. The capillary size was 47 μm with a flow rate of approximately 0.5 mL/min of 100 ppm aqueous calcium.

As can be seen from the top photograph, the droplets enter the flame at an angle slightly above horizontal. The hot flame gases sweep the droplets in the vertical direction and desolvation begins. Near the top section of the flame can be seen the emission from calcium. If the diameter of the droplets are measured and compared to the introduction rate, the rate of desolvation can be calculated.

The signal obtained from this method of introduction is shown in the bottom photograph. Each peak represents the emission from one drop of calcium solution. The signal between peaks represents the flame background. Thus a signal obtained with widely spaced droplets could be used to

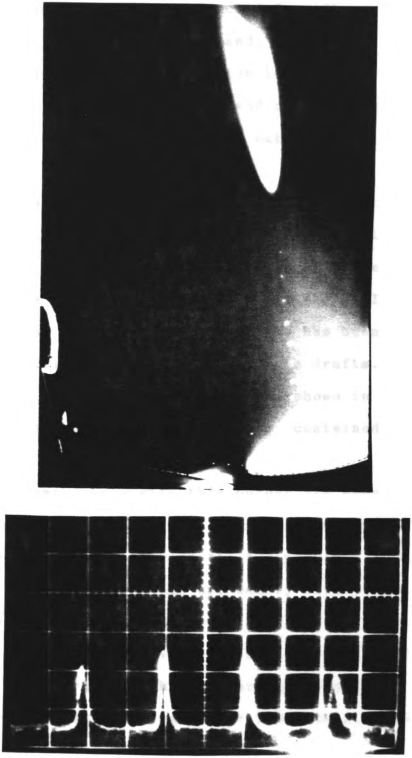


Figure 70. Top: Droplets being introduced into a flame. Bottom: Signal obtained from droplet introduction. Vertical axis: relative signal. Horizontal axis: 0.015 ms/div.

obtain the sample emission corrected for flame background. If the droplet rate is increased, eventually the emission peaks merge and continuous emission is obtained. For this combination of production rate and droplet size continuous emission was obtained at about one out of every 100 droplets introduced.

In an effort to improve the reproducibility of droplet emission even further, a new burner was designed. This burner incorporates the best features of the Meeker burner, but can use a hotter air-acetylene flame. In addition, provision for a nitrogen sheath gas has been provided to further stabilize the flame against room drafts. The flame gas settings and flame appearances are shown in Table 14. A diagram of the burner construction is contained in Figure 71.

Preliminary work with this burner has been successful. Emission can be observed with larger droplet sizes than could be used in either of the two previous burners. The characteristics of the flame are easily altered to suit the particular type of droplet stream used. The nitrogen sheath gas has been shown to provide an effective shield against moderate room drafts. This burner should provide a stable, well-characterized environment for flame/droplet work in the future.

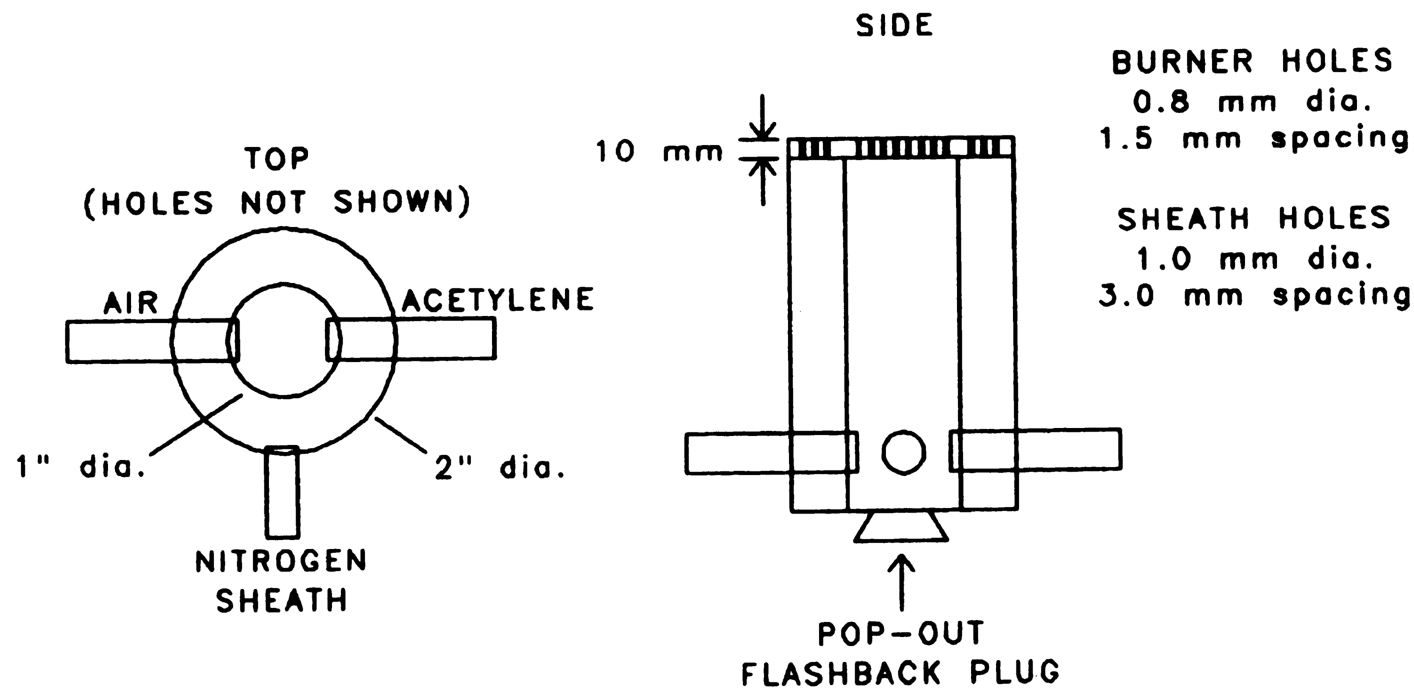


Figure 71. Design of sheathed air-acetylene Meeker burner for isolated droplet emission spectroscopy.

Table 14. Gas flows used for IDG burner.

Fuel(L min^{-1})	Air(L min^{-1})	Height(in)	Appearance
1.0	17.0	7	cone shaped, oxidizing
1.2	17.0	8	cylindrical, oxidizing
1.5	17.0	9	blue outer, cyl., ox.
2.0	17.0	12	blue outer, cyl., ox.
2.5	17.0	14	cylindrical, oxidizing
1.0	14.5	6	cone, oxidizing
1.5	14.5	9	cylindrical, oxidizing
2.0	14.5	12	cylindrical, reducing
2.5	14.5	14	cylindrical, luminous
1.0	12.5	6	cone, oxidizing
1.5	12.5	10	cylin., stoichiometric
2.0	12.5	12	cylindrical, luminous

VI. CONCLUSIONS AND FUTURE PERSPECTIVES

In this research dissertation, two instruments have been presented which can aid the analytical chemist in the separation and characterization of complex mixtures. Several unique approaches have been utilized in the instrumental design which make the intruments both powerful and easy to use. These design initiatives are consistent with current research trends and represent a significant contribution to modern analytical instrumentation.

As in previous chapters, the multidimensional HPLC is discussed first. Important features of the instrumental design are briefly summarized, and conclusions drawn from the performance data are presented. Suggestions for future research using the instrument are also given.

The important features of the isolated droplet generator are then summarized, its probability for success as an HPLC interface is evaluated, and several recommendations for future applications are outlined.

A. Multidimensional HPLC

The multidimensional HPLC described in this dissertation has been successfully used for a wide variety of column switching and backflushing applications. Several design factors can be identified which have significantly contributed to this success.

Detector-based event control was shown to improve significantly the precision of valve switching and synchronous parameter changes. Lack of such precision was one of the biggest problems preventing widespread use of the technique. Time-based sequencing had been used successfully, but the event times had to be determined on a trial-and-error basis. Run-to-run precision was shown to be quite poor for this method. Since the day-to-day variation in retention time is even greater, development of a consistent analytical method with valve switching based solely on time was very difficult, if not impossible. A well written detector-based event control strategy can adapt to changes in retention time and thereby maintain the correct chromatographic relationship. In addition, it is possible to detect the presence or absence of various peaks and alter the event sequencing accordingly. Many other conditional event control sequences are possible using this approach. Indeed, the implementation of detector-based event control opens a new dimension of creativity in multidimensional chromatography.

Another factor which has prevented the technique from becoming more popular was the lack of an integrated instrument. In the past, separate sequencers or timers were used for each valve and had to be synchronized by hand. Even when multiple channel timers became available, solvent delivery was asynchronous with valve switching. Centralized computer control, when coupled with an intelligent solvent delivery system, permits very detailed separation schemes to be employed where all events are synchronized. Such events can include solvent changes, flow rate changes, starting a gradient or even custom gradient construction through the use of solvent composition stepping. Other parts of the instrument can be controlled as well. The scale factor with which the data are plotted or the data taking rate can be altered using the same commands which control valving events. Such integration is necessary for truly automated operation.

Perhaps the most important factor which has contributed to the success of this instrument is the development of an easy method for generating and saving the separation schemes for each experiment. Although centralized computer control is possible with any computer language, some are better suited to instrument control than others. The choice of the FORTH operating system for implementation of the system commands allows the user a choice of either entering a line of commands directly, interactively compiling a new command from existing words, or loading a previously defined command sequence from disk. The language is much more readable than machine code, faster than BASIC, and more easily modified than FORTRAN. The basic operation of the instrument is easily learned by novice users, yet the language is powerful enough to handle very advanced programming techniques. Thus, the "user interface" can adapt to a wide variety of resercher's needs and preferences.

The applications presented show only a small portion of possible valving configurations. These do demonstrate the capabilities of MHPLC, however, as well as some of the chromatographic requirements for successful implementation. Several observations are immediately apparent. First, most MHPLC separations require more extensive solvent manipulation than traditional HPLC. This is especially true if mode sequencing or stationary phase programming is being employed. Some proponents of MHPLC have claimed that column switching techniques can completely replace gradient separations. This may be true in certain cases, but this research did not find such a statement to hold true in general. Multidimensional HPLC is best thought of as a way to increase the power of gradient separations, not as a way to replace them. The cases where isocratic operation can be successful are in backflushing applications or heartcutting to a column of the same stationary phase.

Many avenues for future research with this instrument are available. Because the instrument operates in a hierarchical computing environment, much more computing power is available than in a stand-alone system. An excellent use of this computing power is in the application of artificial intelligence methodology to separation optimization. Simplex optimization of isocratic separations is a feature available on many commercial HPLC systems. This approach to optimization requires that several experiments be performed to define the initial simplex. With the added computing power of a minicomputer, an expert system could be developed which could predict the initial separation parameters based on sample information. Optimization of gradient separations or even multidimensional separations may also be possible. The hardware for a closed-loop optimization scheme is already in place. The microcomputer would function as an instrument controller and data acquisition device. The minicomputer would analyze the data, evaluate the separation, write a new separation program in the FORTH control language and send a command to the microcomputer to run the computer-generated separation. This could be a very exciting research project for someone interested in artificial intelligence as applied to chromatography.

A possibility for future work in the valve-switching area is the use of the instrument to perform infinite-length column separations by using heartcut recycling. This could be accomplished by the valving diagram shown in Figure 72. In this diagram, heartcuts are taken from column 1 onto column 2. Column 2 is then developed and the resulting cut is recycled back onto column 1. This process can be repeated indefinitely. For example, the sample is injected with both valves in the position shown. Solvent is flowing through column 1 only. When the detector senses the desired point in the chromatogram, valve 2 is switched, and a cut is placed on column 2. Valve 2 is then returned to the original position, and column 1 is allowed to clear. Then both valves are switched, and the cut from column 2 is recycled to column 1. Both valves are then returned to the positions shown, and the process is repeated. A detector capable of withstanding the column backpressure would be very helpful in this application, since it could be put at position A and the entire chromatogram detected at each recycle.

B. Isolated Droplet Generation

The isolated droplet generator presented represents the most versatile form of this instrument constructed to date. While the stand-alone version is certainly capable of producing droplets in a variety of frequencies and size ranges, computer control enables the user to manipulate the droplet stream to a much greater degree. In addition, computer control enables the droplet stream to be stabilized

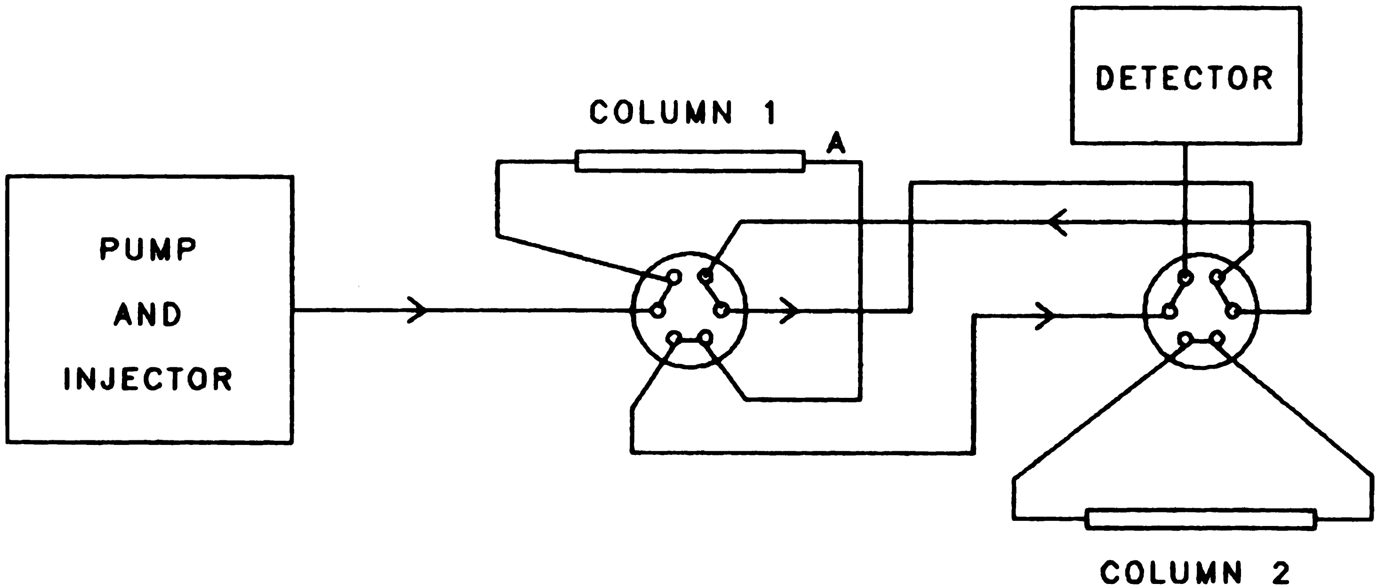


Figure 72. Configuration used for heartcut recycle chromatography.

much more easily by allowing more precise control of the droplet production and charging waveforms.

The fundamental studies with the HPLC as a liquid delivery source for the droplet generator are encouraging enough to support continued research in this area. It should be possible to produce droplets with all common HPLC solvents. Initial results with selected solvents show that both surface tension and viscosity affect the speed with which the jet coalesces into a monodisperse droplet stream. However, these effects are reproducible and can be compensated. Droplet charging of nonaqueous solvents was not possible for all solvents, but should work for most reverse-phase HPLC mobile phase systems. For those cases where the entire droplet stream can be sent to the excitation source (or other experimental destination), all common HPLC solvents should be usable.

The droplet generator instrumentation was constructed to be versatile enough to act as a general-purpose high precision sub-nanoliter liquid handling system. Because of this versatility, many uses of the droplet generator in future research can be envisioned.

Some work has already been done using the droplet generator as a micro-titrator or micro-pH stat (103-105). The computer-controlled version of the droplet generator could easily be modified to do this type of work. In addition, a feedback loop could be established between the device detecting the endpoint (or pH) and droplet control. Thus, the rate of introduction of droplets into the titration vessel could be automatically altered on the basis of the state of the titration.

With the advent of high performance TLC plates, coupling of column HPLC and HPTLC becomes an interesting way to do multidimensional chromatography. The droplet generator could be used in this regard, both to select a portion of the column effluent to be sent to the TLC plate and as a means of automatically spotting the plate at the same time. An application which could be implemented in this manner is the coupling of reversed-phase HPLC to normal-phase HPTLC. This type of mode sequencing is very difficult to do with multidimensional column HPLC because the types of solvent used in reversed-phase chromatography usually deactivate normal phase columns. By using the droplet generator to spot the HPTLC plate with reversed-phase solvent and allowing the solvent to evaporate, normal-phase development could be accomplished without solvent incompatibility problems.

Another use of the droplet generator in HPLC may be as a possible detector based on droplet charging. The nonaqueous solvent charging data presented earlier shows that the solution characteristics drastically affect the ability to charge a droplet. Since charging of the droplets represents a current drain from the charging electrode, a pico-ammeter connected in series with the electrode may be able to detect a change in the solution characteristics as a peak elutes. This detector would probably be similar in selectivity to a conductivity detector, but may exhibit lower detection limits.

One final application of the droplet generator which may prove beneficial is as a liquid introduction technique for laser induced breakdown spectroscopy (LIBS). LIBS is a technique which uses a sharply focussed laser beam to generate a plasma which can be used as an excitation source for atomic emission. Normally, temperatures in the plasma are as high as 10,000-100,000°K, although the region of the plasma is quite small. The droplet generator could be used to position a droplet in the plasma region. If a pulsed laser is used to form the plasma, and if the laser could be triggered by the droplet generator, the phasing of droplet introduction with plasma formation could be optimized for maximum signal. Some initial studies have been done using droplet introduction without phasing, and the results were encouraging (124). Although the precision of the signal was quite poor, when a droplet was in the right position for plasma formation, observed emission was very intense. With synchronized laser triggering and better data acquisition electronics, IDG-LIBS could be a useful analytical technique.

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APPENDIX A

FORTH

REFERENCE

LIST

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Printing time: 15:30:04
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#7	Constant	11
#8	Constant	11
#9	Constant	11
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#>KEYS	Colon	12
#PEAKS	Create	25
%A	Constant	11
%B	Constant	11
%C	Constant	11
%FSCHG	Colon	30
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'BUF	Variable	68
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-A/D	Colon	30
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.DDISK	Colon	22
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3DATA	Variable	24

WORD	TYPE	BLOCK
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ACCEPT	Colon	10
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ADJ	Colon	64
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RESET	Constant	13

WORD	TYPE	BLOCK
DDISK	Colon	68
DEL	Create	21
DEL	Create	66
DELAY	Colon	10
DELAY	Variable	21
DELI	Constant	12
DELSC	Variable	61
DIGREC	Colon	20
DISP	Constant	11
DLY	Create	67
DO2BACK	Colon	23
DO3BACK	Colon	23
DPORT	Constant	13
DRG	Colon	15
DSPEC	Create	28
DT	Colon	27
DTFC	Colon	61
DTI	Colon	27
DUMPER	Colon	9
EDFI	Constant	12
EDITMODE	Colon	19
EDRU	Constant	11
EHELP	Colon	17
EMARK	Variable	32
ENDDATA	Colon	29
ENTE	Constant	11
ENTER	Colon	14
ENTER#	Colon	14
EVENT	Colon	32
FASTDT	Colon	3
FFLAG	Create	22
FLOW	Constant	11
FLOW?	Colon	14
FLOWST	Colon	26
FREE	Colon	4
FTDC	Colon	63
GET#	Colon	12
GETCHR	Code	4
GETFIRST	Colon	36
GETIT	Colon	36
GLDT	Colon	67
GO	Colon	61
HEAD	Colon	27
HELP	Colon	32
HIPUL	Variable	61
HOLD	Constant	11
HT	Colon	2
I#PEAKS	Colon	25
IDG	Colon	63
IDG	Colon	9
IN	Colon	27
INGLDT	Colon	67
INIT	Constant	11
INITBUFF	Colon	22
INITBUFF	Colon	68
INITDAT	Colon	36
INITI	Colon	31

WORD	TYPE	BLOCK
REST	Colon	21
REST	Colon	66
RESUME	Code	1
REVERSE	Colon	2
RFLAG	Variable	24
RNAME	Colon	28
RPT	Colon	17
RPUNC	Variable	6
RST75	Code	13
RSX	Colon	28
RT.	Colon	6
RUGR	Constant	11
RUN	Colon	18
RUNIDG	Colon	62
RUNMODE	Colon	19
S	Colon	7
S12	Code	18
SAVE	Colon	35
SCCW	Colon	38
SCW	Colon	38
SEC	Colon	2
SELECT	Colon	14
SELECT	Colon	38
SETDELAY	Colon	21
SETEMUP	Colon	61
SETTIME	Colon	25
SETUP	Colon	13
SHOW	Colon	2
SLOWDT	Colon	3
SREST	Colon	66
SREST	Colon	21
START	Colon	5
START	Variable	67
STARTEMUP	Colon	61
STARTIDG	Colon	62
STARTTFI	Colon	66
STFI	Constant	12
STORE	Colon	69
STP	Constant	11
STS	Constant	18
STURN	Colon	38
STY	Colon	39
SUSPEND	Colon	29
SW	Colon	21
SYNC	Colon	25
SYNC	Colon	16
SYS	Colon	27
SYSTIM	Colon	4
TAKEDATA	Colon	23
TALK	Colon	2
TEST	Constant	11
TFI	Colon	16
TIME	Constant	11
TIMEOUT	Colon	21
TOGGLE	Colon	21
TURN	Colon	14
UPBL	Colon	28

WORD	TYPE	BLOCK
UPDDISK	Colon	28
UPLIM	Create	67
UPNUM	Colon	28
UPTIM	Colon	4
V#?	Colon	35
VALVE	Colon	26
VIEW	Colon	27
VTAB	Variable	31
W	Colon	14
W<TFI>	Colon	37
WAIT	Colon	21
WFPIP	Colon	35
XXX	Colon	9
XXX	Colon	69
XXX	Colon	69
Z#PEAKS	Colon	25
ZER	Colon	20
ZERO	Colon	20
[?PEAK]	Colon	24
\/	Constant	12

Block Number: 1

```
( MULTITASKING WORDS)

: BACKGROUND CREATE >R OVER + DUP HERE + 2+ 2+ , SWAP
  HERE + 2+ , R> + ALLOT ;

: BUILD OPERATOR @ OVER @ 96 MOVE 2@ DUP OPERATOR
  @ 1+ ! 5 + ! ;

HEX CODE RESUME H POP M E MOV H INX M D MOV XCHG
F7 # M MOV NEXT JMP   DECIMAL
```

Block Number: 2

```
( COMMON UTILITIES)

( SHOW: BEG BLK #,END BLK # --  [CNTRL/Z=STOP, <CR>=NEXT BLK] )
: SHOW 1+ SWAP DO I DUP CR ." BLK #" . CR
      LIST I 1+ BLOCK DROP KEY 26 = IF LEAVE THEN LOOP ;
: 0> MINUS 0< ;
: REVERSE ( n--> , 0=all) DUP 0> NOT IF DROP DEPTH ELSE DUP
DEPTH > IF ABORT" BAD ARG" THEN THEN 1+ DUP 0 DO PAD I 2* + !
LOOP PAD @ 1 DO PAD I 2* + @ LOOP ; ( REVERSES STACK)
: NOBRO UPCR WPRMPT UP" SET /NOBRO=TI:" UPCR ;
: BRO UPCR WPRMPT UP" SET /BRO=TI:" UPCR ;
NOBRO

: TALK BRO WPRMPT TALK ;
: SEC CR DUP . ." second delay ..." 0 DO 14230 0 DO LOOP LOOP ;
: HT L 0 VV ;
```

Block Number: 3

```
( IDG LOAD BLOCK)
10 LOAD ( MULTITASKING WORDS)
15 LOAD ( 9513 WORDS)
61 65 THRU ( IDG WORDS)
: SLOWDT
66 LOAD ( DATA TAKING COUNTER WORDS)
18 LOAD ( ADC BASIC ROUTINES)
22 LOAD 23 LOAD ( DATA TAKING ROUTINES)
28 LOAD 29 LOAD ( DATA TAKING CONTROLLING WORDS) ;
: FASTDT 67 69 THRU ;
: ?DT ." Enter F for fast data taker, S for slow?" KEY
CR DUP 70 = IF FASTDT THEN 83 = IF SLOWDT THEN ;
?DT
```

Block Number: 4

```
( GET SYSTEM TIME AND DATE AND PUT ON STACK)
CODE GETCHR BEGIN U1CMD LDA 2 # ANA 0= NOT END
   U1DATA LDA A L MOV 0 # H MOV HPUSH JMP
: .UP ." TIM " ;
: UPTIM ['] .UP 3 + 4 UPCHAR ;
: NOW UPTIM 13 U1DATA C! 24 0 DO GETCHR LOOP
 24 REVERSE 2DROP 2DROP 2DROP ;
: SYSTIM NOW 18 0 DO EMIT LOOP CR ;
: FREE 30238 HERE - ;

: BYE BYE TALK ;
: ?CREATE  >IN @  -' NOT IF  HERE COUNT TYPE
   ."  isn't unique " CR THEN DROP  >IN !  (CREATE) ;
 ' ?CREATE 'CREATE !
```

Block Number: 5

```
( MEMORY DUMP  USE: START [add --> ])
( Cntrl/Z=EXIT,<CR>=FORWARD ONE SCREEN, <SP>=BACK SCREEN)
( F=FORWARD CONTINUOUS, B=BACK CONTINUOUS, ANY KEY STOPS)

VARIABLE ADD
: NEXT CR 23 0 DO ADD @ I + DUP C@ SWAP ." Address: "
  . DUP 9 EMIT 9 EMIT ." Value: " . DUP 32 > SWAP DUP 127
 < ROT AND IF 9 EMIT 9 EMIT ." Ascii: " EMIT
  ELSE DROP THEN CR LOOP ADD @ 23 + ADD ! ;

: START ADD ! BEGIN NEXT KEY DUP 26 = IF
  ELSE DUP 32 = IF DROP ADD @ 46 - ADD ! 0
  ELSE DUP 66 = IF DROP BEGIN ADD @ 46 - ADD ! NEXT
   ?TERMINAL END 0
  ELSE DUP 70 = IF DROP BEGIN NEXT ?TERMINAL END 0
THEN THEN THEN THEN END ;
```

Block Number: 6

```
( NEW DEFINITION FOR NUMBER)
VARIABLE RPUNC
: RT. 0 RPUNC ! 15 0 DO DUP I + C@ DUP 32 = IF RPUNC @ IF
 I RPUNC @ - RPUNC ! THEN LEAVE
 ELSE DUP 58 = IF I RPUNC ! THEN DUP 44 48 WITHIN IF I RPUNC !
 THEN THEN DROP LOOP RPUNC @ IF -1 RPUNC +! THEN ;

: NEWNUM RT. (NUMBER) ;

' NEWNUM 'NUMBER !
```

Block Number: 7

```
( SEARCH ACROSS BLOCK BOUNDARIES)
( TO USE: endblk S xxx  SEARCHES FROM CURRENT TO endblk)

EDITOR DEFINITIONS

: S #F STRING DROP BEGIN  -FOUND
 IF SCR @ DUP . 1+ DUP BLOCK DROP SCR ! TOP
 ELSE LINE SCR @ . CR THEN
 ?TERMINAL IF DUP 1+ SCR ! THEN
 DUP SCR @ < END DROP ;

FORTH DEFINITIONS
```

Block Number: 8

Block Number: 9

```
      ( [65,67]MICRO.FTH DISK DIRECTORY)

( COMMON UTILITIES) 2 LOAD 4 LOAD
: DUMPER 5 LOAD ; ( MEMORY DUMP)
: MULTITASKING 1 LOAD ;
: LC 6 LOAD 10 32 THRU 35 37 THRU ; ( HPLC CONTROLLER)
: IDG 3 LOAD ;
: XXX ;
```

```
Block Number:  10
( BEGIN MHPLC CONTROLLING ROUTINES)

: DELAY 0 DO 14230 0 DO LOOP LOOP ; ( n --> )
1 LOAD
CREATE '3STOP 1 C,
50 50 50 BACKGROUND 1BACK 1BACK BUILD
50 50 50 BACKGROUND 2BACK 2BACK BUILD
50 50 50 BACKGROUND 3BACK 3BACK BUILD
50 50 50 BACKGROUND 4BACK 4BACK BUILD

: ACCEPT DUP PAD SWAP EXPECT 1+ 0 DO I PAD + C@ 0= IF
  32 I PAD + C! THEN LOOP PAD 1- NUMBER ; ( n -->n2)

' (CREATE) 'CREATE ! ( TURN OFF NOT UNIQUE MESSAGE)


Block Number:  11
( SP8700 KEYBOARD CONSTANTS)  DECIMAL
 96 CONSTANT CLEN    114 CONSTANT CHTI
112 CONSTANT #4        4 CONSTANT #9
120 CONSTANT #5       24 CONSTANT #8
100 CONSTANT #6       16 CONSTANT #7
 50 CONSTANT INIT      0 CONSTANT MAPR
 42 CONSTANT RUGR      8 CONSTANT FLOW
 34 CONSTANT HOLD     20 CONSTANT %C
 52 CONSTANT CONT      2 CONSTANT %B
 40 CONSTANT PURG     10 CONSTANT %A
 32 CONSTANT STP      18 CONSTANT TIME
 48 CONSTANT #1       12 CONSTANT EDRU
 56 CONSTANT #2       36 CONSTANT #3
 76 CONSTANT TEST     74 CONSTANT DISP
 66 CONSTANT PRES     80 CONSTANT #0
 88 CONSTANT #.       68 CONSTANT ENTE

Block Number:  12
( SP8700 KEYBOARD CONSTANTS, CONTINUED)
106 CONSTANT EDFI     116 CONSTANT DELI
 98 CONSTANT STFI     104 CONSTANT \/

CREATE #>KEY #0 C, #1 C, #2 C, #3 C, #4 C, #5 C,
             #6 C, #7 C, #8 C, #9 C,

( CONVERT AN UNSIGNED 16-BIT NUMBER TO ONE KEY CODE PER DIGIT)
: #>KEYS PUNCT @ IF DROP THEN DUP <# #S #>
  DUP ROT ROT 0 DO DUP I + C@ 48 -
  PAD I + C! LOOP DROP 0 DO PAD I + C@ #>KEY + C@ LOOP
  RPUNC @ IF RPUNC @ 0 DO PAD I + C! LOOP #.
  0 RPUNC @ 1- DO PAD  I + C@ -1 +LOOP THEN ;

: GET# 6 ACCEPT #>KEYS ;

```

```
Block Number:  13
  ( BUTTON PRESSER WITH RIM CHECK) DECIMAL 8 BASE !
  177000 CONSTANT APORT
  177002 CONSTANT CPORT
  177003 CONSTANT DPORT
     212 CONSTANT CWORD ( A->, B<-, CLOW->, CHI<- )
      37 CONSTANT RESET ( BASE 2-- 00011111)
   DECIMAL

  CODE RST75 RESET # A MOV SIM NEXT JMP

  CODE READ 0 # H MOV RIM A L MOV HPUSH JMP
  : SETUP CWORD DPORT C! 1 APORT C! ; SETUP
  : PRESS  RST75 DUP APORT C! 2000 0 DO LOOP  READ 64 AND
   IF . ." Acknowledged "
   ELSE 1 APORT C! . ABORT" Not Acknowledged -- Run aborted "
   THEN CR 1 APORT C! 1500 0 DO LOOP ;
```

```
Block Number:  14
  ( VALVE TURNING ROUTINE    VALVE # --  )
  : TURN CPORT C! 5000 0 DO LOOP
      CPORT C@ DUP 16 / SWAP 15 AND -
       ABORT" VALVE FAILURE -- RUN ABORTED" ;

  : SELECT ROT 0 DO  2 * SWAP DUP ROT + LOOP 2 / ;
  : MAKEOUT SELECT CPORT C@ 16 / CR ;
  : CCW DUP 0 1 MAKEOUT OR TURN DROP
   ." Valve #" . ." switched CCW" CR ;
  : CW DUP 1 254 MAKEOUT AND TURN DROP
   ." Valve #" . ." switched CW" CR ;
  : CAM RST75 BEGIN READ 32 AND END ; ( Delay till cam marker)
  : ENTER ENTE 0 REVERSE DEPTH 0 DO PRESS LOOP ;
  : ENTER# #>KEYS ENTER ;
  : FLOW? RST75 BEGIN READ 16 AND END 7 EMIT ." Flow ready" CR ;
  : W CR ." Wait for beep... " ;
```

```
Block Number:  15
  ( CLOCK ROUTINES - CONSTANTS & BASICS)
  OCTAL
    177771 CONSTANT COMAND      ( 9513 command register address)
    177770 CONSTANT DATAREG     ( 9513 data register address)
  DECIMAL

  : CMD  ( N -> ) COMAND C! ;  ( send data to command register)
  : DRG  ( N -> ) DATAREG C! ; ( send data to data register)
  : <DRG (  --> N) DATAREG C@ DATAREG C@ 256 * + ;
  : 2BYTES ( n--> cHI,cLO) DUP 0< IF DUP 32767 AND 256 / 128  +
   ELSE DUP 256 / THEN SWAP 255 AND ;
  : >DRG ( N--> ) 2BYTES DRG DRG ;

  HEX : RESET FF CMD 5F CMD 17 CMD ; DECIMAL
  ( NOTE--RESET LEAVES D.P. AT MM REGISTER) RESET
```

Block Number: 16

```
( 9513 SETUP FOR SECONDS ELAPSED) BINARY
 : 1R 11111111 CMD 01011111 CMD ( Initialize)
00010111 CMD ( MM reg) 11110000 DRG 11001010 DRG ( TOD,BCD div)
00000011 CMD ( #3 mode reg) 00100001 DRG 00001110 DRG ( mode D)
00001011 CMD ( #3 load reg) 01100100 DRG 0 DRG ( = 100.)
00000100 CMD ( #4 mode reg) 00001000 DRG 0 DRG ( mode A)
00001100 CMD ( #4 load reg) 0 DRG 0 DRG ; DECIMAL
: INJST CPORT C@ 128 AND NOT ; ( 1=Inject, 0=Load)
: INJECT CR INJST IF ." Load sample" BEGIN 1 DELAY
  INJST 0= END CR THEN ." Inject sample" CR BEGIN 1 DELAY
  INJST END 108 CMD ( load & arm counters 3 & 4) ;
: <TFI> 168 CMD ( save #4) 20 CMD <DRG (  --> 10ths secs) ;
: TFI <TFI> 1 10 */MOD SWAP DROP ( secs from inj) ;
: SYNC CR INJST IF ." Load sample" BEGIN 1 DELAY INJST 0= END
  THEN CR ." Ready to inject?" KEY W DROP CAM 108 CMD RUGR PRESS
  CR ." Inject now" BEGIN 7 EMIT 1 DELAY INJST END CR ;
```

Block Number: 17

```
( EVENT CONTROLLER)
: CLR 27 EMIT 69 EMIT 27 EMIT 106 EMIT ( clear screen) ;
: POS 31 + >R 31 + R> 89 27 4 0 DO EMIT LOOP ( col,row--> ) ;
: PRNMIN DUP 60 MOD SWAP 60 /
  DUP 10 < IF 32 EMIT THEN . 8 EMIT 58 EMIT
  DUP 10 < IF 48 EMIT THEN . ( sec--> ) ;
: RPT 0 DO DUP EMIT LOOP DROP ;
: BOX 27 EMIT 70 EMIT 70 1 POS 102 EMIT 97 6 RPT 99 EMIT
  70 2 POS 96 EMIT 77 2 POS 96 EMIT 70 3 POS 118 EMIT 97 6 RPT
  116 EMIT 70 4 POS 96 EMIT 77 4 POS 96 EMIT 70 5 POS 101 EMIT
  97 6 RPT 100 EMIT 27 EMIT 71 EMIT CR ;
: ABORTRUN CLR ABORT" ABORTED" ;

: EHELP CLR 11SPEC C@ 77 = IF 34 LIST
  ELSE ." Help unavailable while taking data." THEN ;

```

Block Number: 18

```
( ADC BASIC ROUTINES)
OCTAL
177700 CONSTANT STS
177710 CONSTANT 0ADC
177711 CONSTANT 1ADC
DECIMAL
CODE R12 1ADC LHLD HPUSH JMP
CODE S12 0ADC STA NEXT JMP
: RUN BEGIN S12 1000 0 DO LOOP R12 16 /MOD 16 U.R CR
  DROP ?TERMINAL END ;
: @DAT S12 R12 16 /MOD SWAP DROP ;
```

Block Number: 19

```
( DAC BASIC ROUTINES)
OCTAL
177720 CONSTANT 1DAC
DECIMAL
: !1DAC 1DAC C! ;

( SP8700 MODE SELECTION)

: EDITMODE RST75 \/ APORT C! 2000 0 DO LOOP READ 64 AND NOT
  1 APORT C! 1500 0 DO LOOP IF EDRU PRESS THEN ;

: RUNMODE RST75 \/ APORT C! 2000 0 DO LOOP READ 64 AND
  1 APORT C! 1500 0 DO LOOP IF EDRU PRESS THEN ;
```

Block Number: 20

```
( DIGITAL RECORDER DRIVER)
VARIABLE OFSET
VARIABLE RANGE
CVARIABLE '1STOP : 1STOP 1 '1STOP C! ;
CVARIABLE MARK : !MARK MARK C! ; 10 !MARK
: ZER 2048 DUP ROT SWAP 100 */ - DUP OFSET !
 4096 SWAP - RANGE ! ; 20 ZER
: ZERO CLR ." % OF FULL SCALE? " 4 ACCEPT ZER ;
: CONDAC OFSET @ - DUP 0< IF DROP 0 THEN 256 RANGE @ */ ;
: .MARK @DAT CONDAC DUP MARK C@ DUP ROT + 255 >
  IF - ELSE + THEN !1DAC ;
: ?MIN BEGIN TFI DUP 0= IF DROP 1 THEN 60 MOD DUP 0= IF .MARK
  THEN PAUSE END ;
: LMARK CLR ." LENGTH OF MARK (2.2MM/UNIT)? " 4 ACCEPT !MARK ;
: DIGREC 0 '1STOP C! 1BACK ACTIVATE BEGIN PAUSE @DAT CONDAC
  !1DAC ?MIN '1STOP C@ PAUSE END STOP ;
```

Block Number: 21

```
( VARIABLE DELAY TIMERS n -->  ; n=100ths secs.)
: 2R 5 CMD ( #5 MODE REG) 0 DRG 15 DRG ( MODE A)
 1 CMD 0 DRG 15 DRG 2 CMD 0 DRG 15 DRG ;
 CREATE DEL 100 , CREATE 'REST 10 , CREATE ARM? 1 C,

: SETDELAY ( n-->) 13 CMD ( #5 LOAD REG) 2BYTES DRG DRG
  112 CMD ( LOAD & ARM #5) 13 CMD 0 DRG 0 DRG ;
: TIMEOUT ( n-->) SETDELAY BEGIN PAUSE
  176 CMD ( SAVE #5) 21 CMD <DRG ( GET #5)  -1 = END ;
: SREST ( n-->) 9 CMD 2BYTES DRG DRG 97 CMD 9 CMD 0 DRG 0 DRG ;
: REST ( n-->) SREST BEGIN PAUSE 161 CMD 17 CMD <DRG -1 = END ;
: SW ( n-->) 10 CMD 2BYTES DRG DRG 98 CMD 10 CMD 0 DRG 0 DRG ;
: WAIT ( n-->) SW BEGIN PAUSE 162 CMD 18 CMD <DRG -1 = END ;
: RESET RESET 1R 2R ; RESET ( REPROGRAMS 9513)
: TOGGLE ARM? C@ IF 196 CMD 0 ARM? C! ELSE 36 CMD 1 ARM? C!
  THEN ;
```

```
Block Number:  22
 ( DATA TAKING BUFFER INITIALIZATION )
CREATE LEN 20 , ( DEFAULT LENGTH IN MINUTES FOR DT INT. CALC.)
: .DDISK ." DFLTDAT.FTH " ;

 VARIABLE 'BUF VARIABLE CPNTR
 CREATE FFLAG 1 C, CREATE '2STOP 1 C, CREATE NOWBLK 1 ,
 : DDISK 11SPEC 25 BLANK ['] .DDISK DUP
   3 + SWAP 2+ C@ 11SPEC SWAP MOVE ;

 : INITBUFF FLUSH EB DDISK  BUFFER 'BUF !
   1 IDENTIFY UPDATE 0 '3STOP C!
   2 NOWBLK ! 4 CPNTR ! -1 'BUF @ ! 'REST @ 'BUF @ 2+ ! ;
: RATE? LEN @ 60 * 100 / DUP 10 < IF DROP 10 THEN 'REST ! ;
: LENGTH ." Chromatogram length in minutes?" 3 ACCEPT CR DUP
  LEN ! RATE? 'REST @ ." DT Int.= " . ." hs" CR ;
RATE?

Block Number:  23
 ( BACKGROUND TASKS FOR DATA TRANSFER )
: DO2BACK 2BACK ACTIVATE BEGIN STOP 0 '2STOP C! 0 FFLAG C!
  BUFFER 'BUF ! NOWBLK @ IDENTIFY 4 CPNTR ! 1 FFLAG C!
  FLUSH UPDATE  1 NOWBLK +! 1 '2STOP C! 0 END ;

: NXTPNT 'BUF @ CPNTR @ + ! 2 CPNTR +! CPNTR @
  1024 = IF BEGIN PAUSE '2STOP C@ END
  2BACK RESUME BEGIN PAUSE FFLAG C@ END
  -1 'BUF @ ! PAUSE 'REST @ 'BUF @ 2+ ! PAUSE
  THEN ;
: DO3BACK 3BACK ACTIVATE BEGIN <TFI> @DAT NXTPNT
  NXTPNT 'REST @ REST  '3STOP C@ END STOP ;

: !PARAM -1 NXTPNT 'REST @ NXTPNT ;
: TAKEDATA INITBUFF DO3BACK DO2BACK ;

Block Number:  24
 ( ***PEAK FINDER*** RFLAG=1,REPORT READY; 1DATA=BASELINE; )
 ( 2DATA=TOP OF PEAK; 1TIME=START OF PEAK; 2TIME=TOP PEAK)
VARIABLE 1DATA VARIABLE 2DATA VARIABLE 3DATA VARIABLE 'TIME
VARIABLE 1TIME VARIABLE 2TIME VARIABLE 3TIME CVARIABLE RFLAG
CVARIABLE 1THRESH CVARIABLE 2THRESH CVARIABLE '4STOP
: MMARK MARK C@ SWAP !MARK 100 0 DO .MARK LOOP !MARK ;
: [?PEAK] BEGIN @DAT TFI 1TIME !
 1DATA ! BEGIN PAUSE @DAT TFI 2TIME ! DUP 2DATA ! 1DATA @
- 1THRESH C@ > DUP NOT IF 2DATA @ 1DATA ! 2TIME @ 1TIME !
THEN 'TIME @ TIMEOUT
 END 5 MMARK BEGIN PAUSE @DAT TFI 3TIME ! DUP 3DATA ! 2DATA @
SWAP - 2THRESH C@ > DUP NOT IF 2DATA @ 3DATA @ - 0< IF
 3DATA @ 2DATA ! 3TIME @ 2TIME ! THEN
THEN END 1 RFLAG C! BEGIN PAUSE RFLAG C@ NOT END '4STOP C@
 END ;
: ?PEAK 0 '4STOP C! 4BACK ACTIVATE [?PEAK] STOP ;
```

Block Number: 25

```
( PEAK FINDER AUXILIARY ROUTINES, #PEAKS=CURRENT PEAK COUNT)
: !THRESH CLR ." BASELINE THRESHOLD (A/D UNITS)?" 4 ACCEPT
  1THRESH C! CR ." PEAK THRESHOLD (A/D UNITS)?" 4 ACCEPT
  2THRESH C! ;
CREATE #PEAKS 0 ,
20 1THRESH C! 40 2THRESH C! 0 RFLAG C! 50 'TIME !
: REPORT 27 EMIT 107 EMIT 1 #PEAKS +!
 ." PEAK START:" 1TIME @ PRNMIN ." MAX:" 2TIME @ PRNMIN
 ." HEIGHT (A/D UNITS):" 2DATA @ 1DATA @ - . 1DATA @ ." BASE:" .
 ." COUNT:" #PEAKS @ . 0 RFLAG C! CR 27 EMIT 106 EMIT ;
: INJECT INJECT 0 #PEAKS ! ; : SYNC SYNC 0 #PEAKS ! ;
: 4STOP 1 '4STOP C! ;
: SETTIME CLR ." 100ths of seconds delay?" 5 ACCEPT 'TIME ! ;
: Z#PEAKS 0 #PEAKS ! ;
: I#PEAKS 1 #PEAKS +! ; : D#PEAKS -1 #PEAKS +! ;
```

Block Number: 26

```
( SYSTEM STATUS REPORT FOR LC)
: RD ." RECORDER DRIVER:" '1STOP C@ IF ." OFF" ELSE ." ON"
THEN CR ." Offset (A/D units):" OFSET @ . CR ." Range (A/D units
):" RANGE @ . CR ." Zero (% neg. volts allowed):" 2048 DUP OFSET
 @ - 100 ROT */MOD SWAP IF 1+ THEN . ." %" CR ;
: PF ." PEAK FINDER:" '4STOP C@ IF ." OFF" ELSE ." ON" THEN CR
 ." Base thresh.(A/D units):" 1THRESH C@ .
 CR ." Peak thresh.(A/D units):" 2THRESH C@ . CR ." Sampling int
erval(100ths of secs.):" 'TIME @ .  CR ;
: DAC @DAT CONDAC ." DAC #1 :" . ."     " ;
: A/D R12 16 /MOD SWAP DROP ." A/D :" . CR ;
: FLOWST ." Flow status:"
 RST75 READ 16 AND IF ." Ready" ELSE ." Not ready" THEN CR ;
: VALVE DUP ." Valve " . ." :" CPORT C@ 8 ROT 0 DO 2* LOOP AND
 IF ." CCW" ELSE ." CW" THEN ."     " ;
```

Block Number: 27

```
( MORE SYSTEM STATUS WORDS)
: HEAD  ." *** S Y S T E M   S T A T U S ***" CR CR ;
: MM ." Marker length (DAC units):" MARK C@ . CR ;
: IN ." Injector position:" INJST IF ." Inject" ELSE
  ." Load" THEN CR ;
: ?#PEAKS ." Current peak count:" #PEAKS @ . CR CR ;
: DT ." DATA TAKING:" '3STOP C@ IF ." OFF"
 11SPEC C@ 68 = IF ."  (SUSPENDED)" THEN ELSE '2STOP C@ IF
." ON" ELSE ." FLUSHING" THEN
 THEN CR ." Block:" NOWBLK @ 1- . CR ;
: DTI ." D.T. Interval:" 'REST @ . ." hs" CR ;
: SYS 27 EMIT 72 EMIT HEAD RD MM CR PF ?#PEAKS DT DTI CR DAC A/D
  1 VALVE 2 VALVE CR FLOWST IN ;
: ?SYS CLR SYS ;
: VIEW '3STOP C@ CLR IF ." Block?" 3 ACCEPT CR LIST ELSE
  ." Can't view a block while taking data." CR THEN ;
```

Block Number: 28

```
( DATA FILE RENAMING ROUTINE)

CREATE DSPEC 25 ALLOT
: RNAME CR ." Enter file name (no .ext)"
  DSPEC 25 2DUP BLANK EXPECT ;
: UPBL UP"  " ;
: UPNUM (.) UPCHAR UPBL ;
: RSX UP" .RSX/R " ;

: UPDDISK ['] .DDISK 2 + COUNT UPCHAR ;

: RENAME DSPEC C@ IF UPCR WPRMPT COMMAND-STR
 UPDDISK SWITCHES  1 UPNUM NOWBLK @ 1- UPNUM
 DSPEC 25 -TRAILING UPCHAR RSX UPCR  THEN 11RESET ;
```

Block Number: 29

```
( MORE DATA TAKING ROUTINES)

: SUSPEND 1 '3STOP C! ;
: MOREDATA !PARAM 0 '3STOP C! DO3BACK ;
: ENDDATA 1 '3STOP C! -1 NXTPNT -1 NXTPNT 1022 CPNTR !
  -1 NXTPNT BEGIN PAUSE FFLAG C@  END
  FLUSH RNAME RENAME ;

: NEWREST ( n-->) CLR DEL @
  ." Enter delay for data taking (" . ." units per second)"
  5 ACCEPT '3STOP C@ 0= IF SUSPEND 'REST ! MOREDATA
  ELSE 'REST ! THEN ;
```

Block Number: 30

```
( EVENT CONTROL BASED ON A/D CHANGE)

: +A/D ( u -- )  ." Event will occur at " DUP . ." A/D units"
  CR BEGIN PAUSE DUP @DAT - 0< END DROP ;

: -A/D ( u -- )  ." Event will occur at " DUP . ." A/D units"
  CR BEGIN PAUSE DUP @DAT SWAP - 0< END DROP ;

: A/DCHG ( n -- ) DUP @DAT + SWAP 0< IF -A/D ELSE +A/D THEN ;

: +%FS ( u -- ) RANGE @ 100 */ OFSET @ + +A/D ;
: -%FS ( u -- ) RANGE @ 100 */ OFSET @ + -A/D ;
: %FSCHG ( n -- ) RANGE @ 100 */ A/DCHG ;
```

Block Number: 31

```
( EVENT'S VECTORED EXECUTION TABLE)
VARIABLE VTAB 50 ALLOT
: NOACTION CLR ." NO SUCH COMMAND" ;
: INITI 26 0 DO I 2* VTAB + ['] NOACTION SWAP ! LOOP ; INITI
: !VTAB ' SWAP 2* VTAB + ! ;
( R) 17 !VTAB DIGREC      ( S) 18 !VTAB ?SYS
( O) 14 !VTAB 1STOP       ( U) 20 !VTAB SUSPEND
( C)  2 !VTAB CLR         ( B)  1 !VTAB TAKEDATA
( A)  0 !VTAB ABORTRUN    ( M) 12 !VTAB MOREDATA
( H)  7 !VTAB EHELP       ( E)  4 !VTAB ENDDATA
( Z) 25 !VTAB ZERO        ( N) 13 !VTAB NEWREST
( L) 11 !VTAB LMARK       ( F) 5  !VTAB D#PEAKS
( T) 19 !VTAB !THRESH     ( I) 8  !VTAB I#PEAKS
( P) 15 !VTAB ?PEAK       ( K) 10 !VTAB Z#PEAKS
( Q) 16 !VTAB 4STOP       ( V) 21 !VTAB VIEW
( D)  3 !VTAB SETTIME     ( X) 23 !VTAB TOGGLE
```

Block Number: 32

```
( EVENT CONTROLLER FRONT-END ROUTINE)
: ?COM KEY DUP 65 91 WITHIN NOT IF DROP 72 THEN
  65 - 2* VTAB + @ EXECUTE ;
CVARIABLE EMARK 20 EMARK C!
: EVENT 27 EMIT 106 EMIT SWAP 60 * + EMARK C@ MMARK
BEGIN ?TERMINAL IF ?COM
 THEN RFLAG C@ IF REPORT THEN
 DUP 71 4 POS PRNMIN DUP TFI DUP BOX 71 2 POS PRNMIN 1 +
< END 27 EMIT 107 EMIT ." Event @ " PRNMIN ." commencing"
CR ( min,sec--> ) ;

: CLOCK 108 CMD ;
: HELP CLR 11SPEC C@ 77 = IF 33 LIST
  ELSE ." Help unavailable while taking data" THEN ; HELP
' ?CREATE 'CREATE ! ( TURN ON NOT UNIQUE MESSAGE)
```

Block Number: 33

```
     WORDS AVAILABLE FOR HPLC AND VALVE CONTROL
PRESS < k -- > Presses one button wth constant k.
ENTER < k1,k2,...kX -- > Presses given keys, then ENTER key.
ENTER# < n or d -- > Converts number to key codes and ENTERs.
INJECT < -- > Waits for load/injection, then starts micro clock.
SYNC < -- > Syncs w/SP8700 by pressing RUGR & cueing for inject.
CCW or CW < n -- > Turns valve n in spec. direction.
EVENT < m,s -- > Delays till min,sec after injection.
WAIT < hs -- > Delays for hs hundreths of seconds.
+%FS or -%FS < n -- > Delays till DAC is n% of full scale
%FSCHG < n -- > Delays till DAC changes by n% of full scale
CAM < -- > Waits till cam marker is sensed.
FLOW? < -- > Waits till "flow ready" is sensed.
HELP < -- > Lists this help frame on the terminal.
CLOCK < -- > Resets micro clock to zero.
See documentation and SCR 30 for sample run.
```

Block Number: 34

```
EVENT CONTROLLER COMMANDS
A -- Abort run                     B -- Begin taking data
C -- Clear screen                  D -- Sets delay for peak finder
E -- End data taking               F -- False peak,decrement count
H -- Lists this info               I -- Increment peak count
K -- Kill peak cnt (set to 0)      L -- Sets length of min. marker
M -- Take more data
N -- New d.t. interval
O -- Turns off recorder driver
P -- Turns on peak finder
Q -- Turns off peak finder
R -- Turns on recorder driver
S -- Displays system status
T -- Sets p.f. threshold values
U -- Suspend d.t. temporarily      V -- View (list) a block
X -- Stop/Restart clock            Z -- Sets recorder zero
```

Block Number: 35

```
( SAVE & RECALL A/D VALUES)
CREATE A/DVALUES 20 ALLOT
: INITIL 10 0 DO 2048 A/DVALUES I 2* + ! LOOP ; INITIL
: V#? 0 9 WITHIN NOT ABORT" Save/recall error -- invalid arg" ;
: SAVE ( n -- ) DUP V#? @DAT SWAP 2* A/DVALUES + ! ;
: RECALL ( n -- d) DUP V#?  2* A/DVALUES + @ ;
( START OF REGRAPHING UTILITY)
: WFPIP BEGIN GETCHR DUP 93 = END DEPTH DEPTH REVERSE
  DROP DROP DEPTH 1- 0 DO EMIT LOOP CR
  70 > ABORT" ABORTED -- FPIP ERROR" ;
```

Block Number: 36

```
( REGRAPHING UTILITY)
 VARIABLE ADRNG VARIABLE ADOFF
: INITDAT FLUSH RNAME  UPCR WPRMPT COMMAND-STR DSPEC 25
 -TRAILING UPCHAR RSX UPBL UPDDISK SWITCHES 1 UPNUM UPCR
  WFPIP EB DDISK ;
: OFF CPORT C@ 16 / 4 OR CPORT C! ;
: ON CPORT C@ 16 / 11 AND CPORT C! ;
: GETIT OFF NOWBLK @ BLOCK 'BUF ! ON ;
: PARAMS?
 ." MINIMUM A/D VALUE TO GRAPH?" 5 ACCEPT CR DUP ADOFF !
 ." MAXIMUM A/D VALUE TO GRAPH?" 5 ACCEPT CR SWAP - ADRNG ! ;
: >DAC ADOFF @ - DUP 0< IF DROP 0 THEN 256 ADRNG @ */
  DUP 255 > IF DROP 255 THEN ;
: GETFIRST 1 NOWBLK ! 1 BLOCK 'BUF ! 0 CPNTR ! ;
: NXT 'BUF @ CPNTR @ + @ 2 CPNTR +! CPNTR @ 1024 = IF
 0 CPNTR ! 1 NOWBLK +! TOGGLE GETIT TOGGLE THEN ;
```

Block Number: 37

```
( REGRAPH UTILITY FRONT END STUFF)

( 9513 SPEED UP )
: 9513SU 11 CMD 10 >DRG 2 CMD 0 DRG 14 DRG ;

: W<TFI> BEGIN DUP 1- <TFI> < END DROP ;

: REGRAPH OFF 0 !1DAC INITDAT PARAMS? GETFIRST ON 9513SU CLOCK
  BEGIN NXT DUP 0< IF DROP
  NXT DUP 0< IF EB 11RESET 7 EMIT 0 !1DAC
  RESET OFF ABORT" <EOF>" THEN DROP NXT
  THEN NXT SWAP W<TFI> >DAC !1DAC 0 END ;
```

Block Number: 38

```
( SLOW VALVE TURNING ROUTINE    VALVE # --  )
: STURN CPORT C! 25000 0 DO LOOP
    CPORT C@ DUP 16 / SWAP 15 AND -
     ABORT" VALVE FAILURE -- RUN ABORTED" ;

: SELECT ROT 0 DO  2 * SWAP DUP ROT + LOOP 2 / ;
: MAKEOUT SELECT CPORT C@ 16 / CR ;
: SCCW DUP 0 1 MAKEOUT OR STURN DROP
 ." Valve #" . ." switched CCW" CR ;
: SCW DUP 1 254 MAKEOUT AND STURN DROP
 ." Valve #" . ." switched CW" CR ;
```

```
Block Number:  61
( PHASE SHIFTED FREQ GENERS. FOR IDG -- BLOCK 1/3)
( TO USE: BIM DIV.,DELAY SC., LOW PULSE, HI PULSE GO)
( UNITS ARE MICROSECONDS, DO SETEMUP ONCE AT START)
VARIABLE BIMDIV VARIABLE DELSC VARIABLE LOPUL VARIABLE HIPUL
: DTFC 10 * 16960 15 ROT M/ 2/ ;
: NF BIMDIV @ DTFC CR CR ." NEW FREQ: " 10 * U. CR ;
HEX
: SETEMUP 17 CMD 5000 >DRG 1 CMD B22 >DRG 2 CMD B02 >DRG
  3 CMD 8B62 >DRG ;
: LOADEMUP ( C1,C2,C3L,C3H--> ) 13 CMD >DRG 0B CMD >DRG
  0A CMD >DRG 9 CMD >DRG ;
: STARTEMUP C7 CMD 47 CMD E1 CMD E2 CMD E3 CMD 27 CMD ;
: GO LOADEMUP STARTEMUP ;
DECIMAL SETEMUP
: CHKFRQ DUP 5000 > IF CR ." *** FREQ TOO HIGH ***"
 7 EMIT DROP 5000 CR THEN ;

Block Number:  62
( PHASE SHIFTED FREQ.GENERATORS FOR IDG -- BLOCK 2/3)
: !VAR ( N,N,N,N--> ) HIPUL ! LOPUL ! DELSC ! BIMDIV ! ;
: .VAR 13 EMIT HIPUL @ LOPUL @ DELSC @ BIMDIV @
 ." BIMORPH DIVIDER="  U. ."  DELAY SCALAR=" U. ."  LOW PULSE ="
 U.   ."  HIGH PULSE =" U.  ;
: CALDIV BIMDIV @ 2* DELSC ! 2DUP 2* 1+ BIMDIV @ * LOPUL !
2* 1- BIMDIV @ * HIPUL ! ;
: STARTIDG BIMDIV @ DELSC @ LOPUL @ HIPUL @ GO ;
: RUNIDG STARTIDG BEGIN KEY DUP 61 = IF LOPUL @ 2 > IF
  1 HIPUL +! -1 LOPUL +! THEN THEN DUP 45 = IF HIPUL @
  2 > IF 1 LOPUL +! -1 HIPUL +! THEN THEN DUP 46 = IF
  1 DELSC +! THEN DUP 44 = IF DELSC @ 2 > IF -1 DELSC +! THEN
  THEN DUP 72 = IF BIMDIV @ DUP 10 > IF 1- BIMDIV ! ROT ROT
CALDIV ROT NF ELSE DROP THEN THEN DUP 76 = IF 1 BIMDIV +!
 ROT ROT CALDIV ROT NF THEN STARTIDG .VAR  83 = END 2DROP ;

Block Number:  63
( PHASE SHIFTED FREQ GENERS. FOR IDG -- BLOCK 3/3)
( TO USE: #HI DROPS , #LO DROPS , FREQ TO 2 DECIMAL PLACES)
( THEN IDG -- ,< AND .> KEYS ARE PHASE SHIFTERS)
( -_ AND =+ ADJUSTS DUTY CYCLE -- S FOR STOP -- OTHERS RESTART)
( H AND L ADJUST FREQ. AND RESET DEFAULTS)

: FTDC DROP CHKFRQ DUP DUP
 16960 15 ROT M/ 2/ 10 / DUP BIMDIV ! DTFC
 BIMDIV @ 1+ DTFC 2DUP
 CR ." LO FREQ:" 10 * U. ."  HI FREQ:" 10 * U.
 SWAP ROT - ROT ROT - > IF 1 BIMDIV +!
 ."     USED LO FREQ" ELSE ."     USED HI FREQ" THEN
 ." :DIV=" BIMDIV @ . CR ;

: IDG ( #HI,#LO,FF.FF) FTDC CALDIV RUNIDG ;
```

Block Number: 64

```
( FREQ. RAMP FOR DROP GEN. )

: ADJ BEGIN KEY DUP 46 = IF -1 BIMDIV +! THEN
  DUP 44 = IF 1 BIMDIV +! THEN
  STARTIDG .VAR
  83 = END ;
: RAMPIDG IDG BEGIN BIMDIV @ 1+ BIMDIV !
  STARTIDG .VAR
  1 SEC ?TERMINAL IF ADJ THEN 0 END ;
```

Block Number: 65

```
( ONE-SHOT DROPLET PRODUCTION)
( USE IDG FIRST TO SET PARAMETERS FOR BIMORPH FREQ., )
( PHASING AND DUTY CYCLE.  THEN SAY n ONE-SHOT, WHERE n IS)
( A DELAY IN HUNDRETHS OF SECONDS)

HEX
: 3PROG 3 CMD 8B42 >DRG ;
DECIMAL

: ONE-SHOT 3PROG 0 DO 142 0 DO LOOP LOOP
  STARTIDG .VAR CR ." Hit a key to exit" KEY DROP SETEMUP ;
```

Block Number: 66

```
( CONFIGURE COUNTERS 4 & 5 FOR DATA TAKING/IDG)

16384 >DRG ( MM REG BY PREVIOUS RESET)
4 CMD 3328 >DRG ( 4 MR DOWN, ONCE, BINARY, F3)
5 CMD 3880 >DRG ( 5 MR UP, ONCE, BINARY, F5) 13 CMD 0 >DRG
CREATE DEL 3906 , CREATE 'REST 100 ,

: SREST ( N-->) 12 CMD ( 4 LD) >DRG
 72 CMD 12 CMD 0 >DRG 40 CMD ;
: REST ( N-->) SREST BEGIN PAUSE 168 CMD 20 CMD <DRG -1 = END ;

: STARTTFI 112 CMD ;
: <TFI> ( --> N) 176 CMD 21 CMD <DRG ;

: CLR 27 EMIT 69 EMIT 27 EMIT 106 EMIT ( CLEAR SCREEN ) ;
```

```
Block Number:  67
 ( GREASED LIGHTING DATA TAKER)

18 LOAD
CREATE DLY 1 ,
VARIABLE START CREATE UPLIM 30236 , ( TOP OF AVAIL. RAM)
: INGLDT HERE DUP  START ! UPLIM @ HERE - 2/ ." # PTS=" . CR
UPLIM @ H ! ;

: GLDT UPLIM @ SWAP DO S12 DLY @ 0 DO LOOP R12 I !
 2 /LOOP ;

: LOOK-SEE HERE START @ DO I @ 16 /MOD SWAP DROP I . .
( I 23 /MOD DROP 0= IF KEY DROP THEN) 2 /LOOP ;
( LOOK-SEE LETS YOU VIEW DATA WITHOUT STORING ON 11/23)


Block Number:  68
( DATA TRANSFER FOR GLDT)

VARIABLE 'BUF VARIABLE NOWBLK VARIABLE CPNTR
: .DDISK ." DFLTDAT.FTH " ;
: DDISK 11SPEC 25 BLANK ['] .DDISK DUP
3 + SWAP 2+ C@ 11SPEC SWAP MOVE ;

: INITBUFF FLUSH EB DDISK BUFFER 'BUF ! 1 IDENTIFY UPDATE
2 NOWBLK ! 6 CPNTR ! -1 'BUF @ ! 0 'BUF @ 2 + !
DLY @ 'BUF @ 4 + ! ;

: NXTPNT 'BUF @ CPNTR @ + ! 2 CPNTR +! CPNTR @ 1024 =
IF ." NOW FLUSHING BLK #" NOWBLK @ 1 - . CR FLUSH
 BUFFER 'BUF ! NOWBLK @ IDENTIFY UPDATE 1 NOWBLK +!
0 CPNTR ! THEN ;
28 LOAD

Block Number:  69
( DATA TRANSFER FOR GLDT)

: STORE INITBUFF HERE START @ DO I @ 16 /MOD SWAP DROP
  NXTPNT 2 /LOOP -1 NXTPNT -1 NXTPNT
." NOW FLUSHING LAST BLOCK" CR FLUSH EB RNAME RENAME
 START @ H ! ;
: PSTORE INITBUFF HERE START @ DO I @ 16 /MOD SWAP DROP
  DUP . I . CR
  NXTPNT 2 /LOOP -1 NXTPNT -1 NXTPNT
." NOW FLUSHING LAST BLOCK" CR FLUSH EB RNAME RENAME
  START @ H ! ;
( TO USE: 1.INGLDT initializes memory. 2. Store delay in DLY)
( 3. GLDT takes data and stores it in memory. 4. STORE ships)
( data up to the 11/23. PSTORE prints on terminal and stores)
( File structure: 12bit #s in 16bit cell, -1 -1 = EOF )
( clear memory with FORGET XXX : XXX ; before next run) : XXX ;
```

Block Number: 70

```
( EXAMPLE OF HOW TO CHANGE DROPLET PARAMETERS IN SOFTWARE)

: TEST-RUN ( #HI, #LO, FF.FF --> )
  IDG ( GENERATE INITIAL PARAMETERS AND THEN CONTINUE)
  ( BY HITTING THE S KEY)
  20 SEC ( 20 SECOND DELAY IN SOFTWARE)
  5 DELSC +!  ( ADD 5 MICROSECONDS ONTO DELAY SCALAR)
  STARTIDG ( AND RESTART 9513) 150 SEC ( ANOTHER DELAY)
  2 LOPUL +! -2 HIPUL +! ( ADJUST DUTY CYCLE BY 2 MICROSECONDS)
  ( MAKING LOW PULSE LARGER AND HIGH PULSE SMALLER)
  STARTIDG 400 SEC (RE-START AND DELAY)
  4 50 12.25 IDG ( GENERATE A WHOLE NEW STREAM WITH 4 HIGH)
  ( AND 50 LOW DROPS AT 12.25 kHz, CONTINUE BY HITTING S KEY)
  100 SEC ( ANOTHER 100 SECOND DELAY)
  ( MORE ADJUSTMENTS, ETC., ETC., ETC.) ;
```

APPENDIX B

An Operator's Guide for the Multidimensional HPLC

APPENDIX B

An Operator's Guide for the Multidimensional HPLC

This appendix is meant to serve three purposes. A concise listing of FORTH commands and their arguments is given as a means for easy reference. Detailed explanations of the commands are contained in the "Software" chapter of this dissertation. Where appropriate, examples are shown. A brief start-up and connection procedure is also given in the event that the equipment should be dismantled. Finally, since research is never as easy as one expects, a short troubleshooting guide will be presented for those nasty "unexpected problems."

A. FORTH Commands

The following notation is used: A word in all capitals is a FORTH command. The effect of the word on the stack is shown with the notation:

(before --> after)

where "before" represents the arguments for the word, and "after" represents any numbers produced by the word. Single length signed integers are represented by the letter "n", double length by the letter "d", unsigned single length by the letter "u", SP8700 key codes by the letter "k", a single byte by the letter "b", and logical flags by the letter "f." Defaults and ranges are designated with the notation:

[default,lower limit:higher limit]

A user input is designated by the symbol "xxx" immediately following the word. The word, stack effect, default/limits (if any) are given, followed by the action and an example where appropriate.

SP8700 Communication

PRESS (k -->) [, :] Simulates one keystroke for the key with code k. Aborts if not acknowledged. Ex: Press edit/run button.

EDRU PRESS

ENTER (k1,k2,...kN -->) [, :] Presses all keys given, in first in first out order. Assumes all numbers on stack are key codes. Aborts if any key is not acknowledged. Ex: Enter a flow of 1.5 mL/min.

FLOW #1 #. #5 ENTER

ENTER# (n or d with valid punctuation -->) [, :] Converts the top number on the stack to key codes representing that number and ENTERs them. Stack should be empty prior to using. Numbers with decimal points are acceptable. Ex: Enter a flow of 1.5 mL/min.

FLOW 1.5 ENTER#

GET# xxx (--> k1, k2, ... kN) [, 0:65535] Waits for up to

6 digit input. Valid punctuation is a decimal point or other FORTH punctuation. Converts the input number to a series of SP8700 key codes. No error checking is performed.

#>KEYS (u --> k1,k2, ... kN) [, 0:65535] Stack equivalent of GET#. Useful for converting an index of an iteration loop to key codes.

EDITMODE (-->) [, :] Places the SP8700 in edit mode. If already in edit mode, has no effect.

RUNMODE (-->) [, :] Places the SP8700 in run mode. If already in run mode, has no effect.

FLOW? (-->) [, :] Delays until the "flow ready" LED is lit.

CAM (-->) [, :] Delays until the "cam marker" LED flashes.

Table B1. Key Code Definitions

Key	Mnemonic	Key	Mnemonic
Change Time	CHTI	Test	TEST
Clear Entry	CLEN	Time	TIME
Continue	CONT	1	#1
Display	DISP	2	#2
Delete Line	DELI	3	#3
Edit File	EDFI	4	#4
Edit Mode/Run Mode	EDRU	5	#5
Enter	ENTE	6	#6
Flow	FLOW	7	#7
Hold	HOLD	8	#8
Initialize	INIT	9	#9
Max. Pressure	MAPR	0	#0
Pressure	PRES	.	#.
Purge	PURG	%A	%A
Run Gradient	RUGR	%B	%B
Store File	STFI	%C	%C
Stop	STP	(down arrow)	\/

Valve Control

CW (n -->) [, 1:2] Turns the valve specified in the clockwise direction. If already in the clockwise position, this word has no effect. If not turned in the clockwise direction within the time-out period, the word aborts. Ex: Turn valve 1 in the clockwise direction.

1 CW

CCW (n -->) [, 1:2] Same as CW, but turns valve n in the counterclockwise direction.

VALVE (-->) [, 1:2] Reports the valve position.

INJST (--> f) [, :] Returns a 1 when the injector valve is in the inject position, a 0 for the load position.

IN (-->) [, :] Reports the injector valve position.

SETUP (-->) [, :] Re-programs the 8255 PIO to the state after the software was first loaded. Sets valves to CCW position.

Timing Words

CLOCK (-->) [, :] Starts or re-starts micro clock.

TOGGLE (-->) [, :] Turns clock on/off without resetting.

INJECT (-->) [, :] Starts micro clock when sample is injected.

SYNC (-->) [, :] Starts micro clock and synchronizes with SP8700 clock by cueing for injection and PRESSing the run gradient button at the proper point in the cam cycle.

TFI (--> n) [, :] Returns the number of seconds since micro clock was started.

<TFI> (--> n) [, :] Returns the number of tenths of seconds since micro clock was started.

RESET (-->) [, :] Re-programs AM9513 to the state after software is first loaded.

WAIT (n -->) [, 1:65535] Delays for n hundreths of seconds. Uses AM9513.

EVENT (n1,n2 -->) [, 1:65535] Delays until micro clock reads n1 minutes and n2 seconds. Allows access to commands listed below while waiting by hitting a key once to get the event controllers attention and a second time to issue the command. If the clock is not updating, the event controller is probably waiting for a command. Displays current time and time of next event in upper right corner of screen. Prints report from peakfinder when needed. Ex: Delay until 5 minutes and 30 seconds from injection, turn valve 2 CW and turn it back to CCW 7 minutes and 45 seconds from injection.

INJECT 5 30 EVENT 2 CW 7 45 EVENT 2 CCW

Table B2. Event Controller Commands

Key	Word	Function
A	ABORTRUN	Abort run, return control to term. handler
B	TAKEDATA	Begin taking data.
C	CLR	Clear screen.
D	SETTIME	Set delay for peak finder.
E	ENDDATA	End a data taking run.
F	D#PEAKS	False peak, decrement peak count.
H	EHELP	Lists event controller commands.
I	I#PEAKS	Increment peak count.
K	Z#PEAKS	Kill (zero) peak count.
L	LMARK	Sets length of minute marker for recorder.
M	MOREDATA	Restarts data taking after a SUSPEND.
N	NEWREST	New data taking interval.
O	1STOP	Turns off recorder driver task.
P	?PEAK	Turns on peak finder task.
Q	4STOP	Turns off peak finder task.
R	DIGREC	Turns on recorder driver task.
S	?SYS	Displays system status.
T	!THRESH	Sets peak finder thresholds.
U	SUSPEND	Suspend data taking temporarily.
V	VIEW	List a block on the terminal.
X	TOGGLE	Stop/Restart the microcomputer clock.
Z	ZERO	Sets recorder zero for recorder driver task.

Event Control Using Detector Output

+A/D (u -->) [, 0:4094] Delays until the detector output is greater than u ADC units. Ex: Turn valve 2 CW when the detector output exceeds 3400 ADC units.

3400 +A/D 2 CW

-A/D (u -->) [, 1:4095] Delays until the detector output is less than u ADC units.

A/DCHG (n -->) [, -4094:+4094] Delays until the detector output changes by the specified number of ADC units. The direction is controlled by making n positive for an increase and negative for a decrease. Ex: Turn valve 2 CW after a decrease in detector output of 30 ADC units.

-30 A/DCHG 2 CW

+%FS (u -->) [, 0:99] Delays until the detector output exceeds u percent of full scale on the chart recorder. Ex: Turn valve 2 CW at 55% of recorder full scale.

55 +%FS 2 CW

-%FS (u -->) [, 1:100] Delays until the detector output is less than u percent of the chart recorder full scale.

%FSCHG (n -->) [, -99:+99] Delays until the detector output changes by n percent of the chart recorder full scale. Direction can be specified as in A/DCHG.

SAVE (n -->) [, 0:9] Reads the current detector output and stores the resulting number of ADC units in register n.

RECALL (n --> n2) [, 0:9] Recalls the ADC units which were stored in register n and places them on the stack for use with +A/D or -A/D.

Data Acquisition and Storage

TAKEDATA (-->) [, :] Initiates a data taking run. Sampling rate controlled by the variable 'REST. Sampling delay is performed by AM9513 STC.

SUSPEND (-->) [, :] Temporarily suspends data taking. Buffers are not flushed. Parameters are not altered.

MOREDATA (-->) [, :] Re-starts data taking after a suspend. Puts a flag in the data file consisting of a -1 followed by the current value of 'REST.

ENDDATA (-->) [, :] Terminates a data taking run. Buffers are flushed. Disk specification is changed back to MICRO.FTH. User is prompted for a file name, and an FPIP command line is sent to transfer the number of blocks acquired from the default data file (DFLTDAT.FTH) which is a FORTH emulator file, to an RSX-compatible file ('username'.RSX).

'REST (variable) [12,1:65535] Data taking delay in 100ths of seconds. The value returned by this variable is used by the word REST, which does the delay. Although values as small as 1 can be used, duplicate times will be obtained with values of less than 10, since <TFI> returns times in tenths of seconds.

LEN (variable) [20,1:32767] Expected chromatogram length in minutes. Used by RATE? to set 'REST.

RATE? (n-->) [, 1:32767] Takes an argument in minutes and uses it to set 'REST to a rate which will use 40 blocks of data in the time specified. This is 80% of the default file space, and represents about 10,200 points.

LENGTH (-->) [, 1:999] Prompts for expected chromatogram length and then does RATE?.

NOWBLK (variable) [, 1:] Returns the current block of data being filled.

CPNTR (variable) [, 1:1024] Returns the current offset into the data buffer. Represents the number of data points taken times 4.

@DAT (--> n) [, :] Puts one datum on the stack.

RUN (-->) [, :] Makes a running display of the current ADC output on the terminal.

<u>Peak Finder</u>

?PEAK (-->) [, :] Activates the peak finding task. Makes a small (5 ADC units) positive spike when the start of a peak is sensed.

4STOP (-->) [, :] Deactivates peak finding task.

#PEAKS (variable) [, 0:32767] Returns the number of peaks sensed since injection or since the variable was last reset to zero. Can be used to trigger an event. Ex: Turn valve 2 counter-clockwise 0.2 seconds after the 25th peak is sensed:

BEGIN PAUSE #PEAKS @ 25 = END 20 WAIT 2 CCW

In the above example, the word PAUSE is used to allow background tasks to execute while the BEGIN-END loop is executing.

Z#PEAKS (-->) [, :] Sets #PEAKS to zero.

I#PEAKS (-->) [, :] Increments #PEAKS by 1.

D#PEAKS (-->) [, :] Decrements #PEAKS by 1.

1THRESH (variable) [20,1:32767] Baseline threshold used for sensing the start of a peak. Smaller values are more sensitive (ADC units).

2THRESH (variable) [40,1:32767] Peak maximum threshold used for sensing the top of the peak. Smaller values are more sensitive (ADC units).

!THRESH (-->) [, :] Prompts for values of 1THRESH and 2THRESH.

'TIME (variable) [50,1:32767] Sampling delay in 100ths of seconds for 1THRESH and 2THRESH.

SETTIME (-->) [, :] Prompts for a value of 'TIME.

Recorder Control

DIGREC (-->) [, :] Activates the digital recorder driver.

1STOP (-->) [, :] Deactivates the recorder driver.

OFSET (variable) [1638,0:4095] Number of ADC units subtracted before the conversion to DAC units is made. Set with the word ZERO.

RANGE (variable) [2458,0:4096] Range of ADC units converted to DAC units. Set with the word ZERO.

ZERO (-->) [20,0:100] Prompts for the percentage of the negative range of the ADC to be displayed on the recorder. Normally the detector signal is slightly less than zero volts, so the default is 20%.

MARK (variable) [10,0:4095] Contains the length of the minute mark used in ADC units.

LMARK (-->) [, :] Prompts for the length of the minute marker.

EMARK (variable) [20,0:4095] Contains the length (in ADC units) used for marking the initiation of the word EVENT.

MMARK (n -->) [, 0:255] Makes a positive mark n DAC units on the recorder output. If the mark would cause the DAC to exceed its upper limit, the mark is made in the negative direction. Useful for marking events.

ON (-->) [, :] Turns the recorder chart drive on by opening a mechanical relay in the micro. See wiring description below.

OFF (-->) [, :] Turns the recorder chart drive off by closing the mechanical relay.

REGRAPH (-->) [, :] Prompts for a '.RSX' file to be plotted on the chart recorder. The vertical axis is set by inputting the minimum and maximum ADC values to graph. If this is not known, the entire range should be plotted first by responding with 0 for the minimum and 4095 for the maximum. Scale expansion is achieved by inputting larger minima and smaller maxima. Scale reduction is achieved by inputting 0 for the minima and a value larger than 4095 for the maxima (but less than 32767). The horizontal scale is set by changing the chart drive speed.

<u>Miscellaneous Non-Standard FORTH Words</u>

HELP (-->) [, :] Displays MHPLC help screen.

EHELP (-->) [, :] Displays MHPLC event controller help screen.

?DISK (-->) [, :] Prints current disk filespec at terminal.

11RESET (-->) [, :] Sets disk filespec to MICRO.FTH.

SHOW (n1,n2 -->) [, :] Useful for listing a range of blocks on the terminal. Arguments are beginning block and ending block. Control Z terminates listing, carriage return lists next block without delay.

REVERSE (n -->) [, 0:255] Reverses the order of the top n items on the stack. A 0 argument reverses the entire stack.

NOBRO (-->) [, :] Sets the terminal line to a "no-broadcast" state, so anyone trying to broadcast a message to the terminal will not mess up micro/11-23 communication.

BRO (-->) [, :] Allows terminal to accept broadcast messages.

TALK (-->) [, :] Allows direct communication with 11/23 as if micro were not there. Terminated with the "BREAK" key. Should be used once before downloading to initialize USARTS. Also does a BRO.

GETCHR (-->) [, :] Gets one character from the 11/23 USART and clears the USART to receive the next character.

FREE (-->) [, :] Displays the number of unused bytes of memory.

Additional Editor Commands

HT (-->) [, :] Displays the entire block and shows the current cursor position.

VV (n -->) [0,0:15] Similar to TECO verify command. 0 VV or just VV types the current line and shows cursor position.

DD (n -->) [1,1:64] Similar to TECO delete command. Accepts negative arguments.

CC (n -->) [1,1:?] Similar to TECO character move command. Moves the cursor n characters. Accepts negative arguments.

LL (n -->) [1,1:15] Similar to TECO line move command. Moves the cursor n lines. Accepts negative arguments.

JP (n -->) [0,0:1024] Similar to TECO jump command. Moves to the absolute cursor position specified.

TF (-->) [, :] Types the contents of the "find" buffer.

TI (-->) [, :] Types the contents of the "insert" buffer.

K (-->) [, :] Switches the contents of the find and insert buffers.

CUT xxx Cuts up to and including the specified text into the insert buffer.

B. Startup Procedure

The MHPLC consists of the following components:

1. SP8700 solvent delivery system
2. Chromatronix 220 absorbance detector
3. 8085-based microcomputer
4. Heath H-19 terminal
5. Heath EU-205-11 strip chart recorder
6. Two 6-port, 2-position Rheodyne valves with actuators

7. Two valve control modules

8. One 5-volt power supply

9. Nitrogen or air tank with regulator

The keypad emulator interface has been installed inside the SP8700. Connections are made to the modules as follows:

1. Connect the PIO port on the micro to the keypad emulator on the back of the SP8700. A grey cable with D-connectors on both ends is used for this purpose.

2. Connect pin 17 (bit 0 port C) of the micro D-connector to valve controller 1 "TTL." Connect the "+5" input of the valve controller to the +5 V power supply.

3. Connect pin 21 (bit 4 port C) of the micro D-connector to valve 1 sensing switch. Connect the other side of the switch to the +5 V power supply.

4. Connect pin 18 (bit 1 port C) of the micro D-connector to valve controller 2 "TTL." Connect the "+5" input of the valve controller to the +5 V power supply.

5. Connect pin 22 (bit 5 port C) of the micro D-connector to valve 2 sensing switch. Connect the other side of the switch to the +5 V power supply.

6. Connect pin 24 (bit 7 port C) of the micro D-connector to the injector valve sensing switch. Connect the other side of the switch to the +5 V power supply.

7. Connect pin 25 (cam marker) of the SP8700 D-connector to

the banana plug on the micro labeled "RST 6.5."

8. Connect pin 24 (flow ready) of the SP8700 D-connector to the banana plug on the micro labeled "RST 5.5."

9. Put detector output switch labeled "RECORDER SPAN" on the 1 mA setting. Short the positive detector output lead to the detector return line using a 2.5 kΩ resistor. Connect the positive lead to the ADC positive input on the micro. Let the return lead float. Connect the case ground (thin grey wire) of the detector to the analog ground on the micro.

10. Connect one input of the micro relay to the chart recorder case ground. Connect the other side of the relay to the chart recorder auxiliary connector number 4, labeled "remote chart control."

11. Connect the DAC ground to the recorder ground. Connect the positive DAC output (green jack) to the positive recorder input (labeled 1V/10 inches).

12. Connect the +5 V power supply ground to the micro digital ground (the DAC ground is suitable).

For reference purposes the SP8700 D-connector pinout is as follows:

1. Disable 74150

2. Select 74150 D

3. Select 74150 C

4. Select 74150 B

5. Select 74150 A

6. Select 74155 B

7. Select 74155 A

8. Acknowledge pulse (from monostable)

12. Flow ready state

13. Cam marker state

25. Ground

Pins not listed are not connected. The microcomputer D-connector pinout is:

1-7: Bits 0-6, port A

8: RST 7.5

9-16: Bits 0-7, port B

17-24: Bits 0-7, port C

25: Digital ground

When the above connections have been made the software is loaded as follows:

1. Turn on the micro and terminal. If an "ok" does not appear on the terminal, hit a carriage return. If an "ok" still doesn't appear make sure the communication switch on the front of the micro is in the "micro" position.

2. Type TALK and a carriage return.

3. The 11/23 should respond with a ">" prompt.

4. Log into the HPLC account. See system manager for password.

5. After login messages have stopped, hit the break key. The terminal should beep and a carriage return should produce an "ok" prompt.

6. Type "9 LOAD" and return. The middle green light on the micro should flash, indicating a block is being loaded.

7. When the "ok" prompt is displayed (approximately 15 seconds) type "LC" and return.

8. The MHPLC software should be fully loaded in about 4 minutes. Block numbers should appear on the terminal as they are processed.

C. Troubleshooting

Most problems with the MHPLC have very simple solutions. Listed below are a few common symptoms which represent problems which have occured in the past. Most are caused by simple forgetfulness, and are easily corrected. During the last year of operation, the instrument was very dependable, and with a little care should stay that way.

SP8700 Communication

Symptom: Microcomputer responds to communication efforts with "xx -- Not Acknowledged."

Causes/Corrections:

1. Toggle switch on back of SP8700 in disable position. Switch should be up for communication.

2. SP8700 refused to accept command. Make sure key codes are correct and in the proper sequence. Check manual for correct parameter entry procedure. The command "EDRU PRESS" should always be acknowledged.

3. PIO may have been reprogrammed. SETUP will restore the chip to the correct state.

4. Cable discontinuous. Make sure plugs are secure. Check for continuity.

Valve Control

Symptom: Microcomputer responds to valve switch attempt with "VALVE FAILURE -- RUN ABORTED."

Causes/Corrections:

1. (Valve does not turn.) Five volt power supply not turned on.

2. (Valve does not turn.) PIO may have been accidentally reprogrammed. Issue a SETUP command and try again.

3. (Valve does not turn.) Try manual switches. If manual positions work, then either the sensing switch/wire is bad, or the wire to the controller is discontinuous. To test the sensing switch, manually turn the valve and use ?SYS to see if it reports the correct valve position.

4. (Valve turns, but too slow.) Try increasing nitrogen

pressure. If 75 psi does not produce a fast enough valve switch, the valve needs to be loosened. See valve instructions to make turning easier.

5. (Valve turns too slow when using autoinjector.) This is normal. Use the special valve-turning words SCCW and SCW in block 38.

Software Loading

Symptom: Blocks cannot be downloaded.

Causes/Corrections:

1. USARTS not initialized. TALK first, check for MCR prompt, then try again.

2. Not logged in.

3. MICRO.FTH file is locked. Unlock the file with the PIP command:

PIP MICRO.FTH/UN

The file will lock if FPIP is aborted.

4. (Block will list, but not load) Check for a zero byte in the block.

5. (Block will list, but not load) Bug in the code causes the compiler to enter warp drive. Correct the code.

D. Adding a Third Valve

The original MHPLC design made provision for control and sensing of up to 4 valves through PIO port C. As presently configured, bits 0 and 1 are used to control valves 1 and 2, and bits 4 and 5 are used for sensing of these valves. Bit 7 is used to sense the position of the injector valve. Since this valve is not automated, however, bit 3 is unused.

Bits 2 and 6 were originally meant to be used for control and sensing of the third valve. When control of the strip chart recorder drive was added, a judgement call was made as to whether it would be best to use bit 2 for recorder control and leave port B entirely unused, or use one bit of port B in the event that a third valve would be added. Since two valves seemed adequate for most MHPLC experiments, port B was left unused so it could be devoted to some other purpose.

To add the third valve, it is therefore necessary to first move the recorder control to port B. The words ON and OFF would then have to be re-written to change the bit in port B that is controlling the relay. These words are fairly simple and can easily be altered.

Control of the third valve is added by connecting bit 2 of port C to the new valve controller module TTL input. Sensing of valve three is accomplished by connecting bit 6 of port C to the sensing switch. The words CW and CCW should then be able to control the new valve when given an argument of 3.

Suggestions for Artificial Intelligence Applications

Normally, the microcomputer issues commands to the 11/23, but the 11/23 cannot asynchronously send a command to the microcomputer. Establishing two-way communication with the 11/23 would be the first step in configuring the system for closed-loop artificial intelligence schemes.

One way of receiving commands from the 11/23 is to program the micro to repetitively check the USARTS for the command. The word to get a single character from the USARTS is GETCHR. The command would then be decoded and acted upon. Probably the easiest way to decode the commands from the 11/23 would be to have the program write the commands in the FORTH language initially. The new commands would be placed in a block and the only characters which would then need to be manually decoded from the USARTS would be the block number. Control is then tranferred to the new command sequence by LOADing the block. The USARTS can be checked in a semi-asynchronous fashion by compiling the code that does the checking as a background task.

APPENDIX C

An Operator's Guide for the Isolated Droplet Generator

APPENDIX C

An Operator's Guide for the IDG

The purpose of this appendix is to provide a concise procedure for the generation of various droplet streams. External electrical connections are listed for each component. A short troubleshooting section is also furnished.

A. Single-droplet Production in the Stand-alone Mode

The IDG consists of the following modules:

1. IDG main electronics module
2. Bertran 205A-10P High-voltage power supply
3. Intersil 7226A Counter/timer/frequency meter (CTFM)
4. General Radio 1531AB Stroboscope
5. Bimorph and electrode mounting assembly
6. Liquid delivery device
7. Bimorph/capillary assembly

These modules should be assembled and tested as follows:

1. Connect the case of the high voltage power supply to a good ground, such as a water pipe.
2. To deflect charged droplets up, connect the positive

high voltage to the top deflection electrode. Connect it to the bottom electrode to deflect charged droplets down.

3. Connect the ground wire of the high voltage power supply to the other deflection electrode.

4. Set the output of the high voltage to 4 kV. Put the meter switch to the "I" position to measure the current. Turn the high voltage on and check the current. After an initial deflection, it should quickly drop to zero. If it doesn't, immediately turn off the power supply and check for a short circuit.

5. If the high voltage appears to be working properly set the meter switch to the "V" position. Turn off the power until the remaining connections are made.

6. Plug in the stroboscope and IDG electronics to grounded outlets.

7. Connect the "Strobe Sync" output on the IDG to the "Input" jack on the stroboscope.

8. Set the strobe dial to one of the "External Input" settings. The low intensity setting can flash faster than the high intensity setting, but droplets are sometimes easier to view with a higher intensity.

9. Plug in the CTFM to the left side of the IDG electronics module using the square keyed plug.

10. Connect the "Frequency Meter" output on the IDG to the "A" input on the CTFM.

11. Set the function switch on the CTFM to "Freq" and the

update period to ".1 sec."

12. Set the following switches on the IDG:

Source A: up (divider A internal)

Latch: down (unlatched) or up (latched)

Source B: up (divider B internal)

Bimorph Driver Source: up (internal- divider A)

Pulse Trigger Source: up (internal- divider B)

Bimorph Driver Amplitude: less than about 5.0

Pulse Amplitude: zero volts (fully counterclockwise)

Strobe Sync: 100 (divide pulsing frequency by 100)

Divider B Adjust: 002 (charge every third drop)

Divider A Adjust: 9.9

Frequency Meter Select: Bimorph Driver In

13. Before connecting the bimorph, test the IDG, strobe and CTFM. With the above parameters set, turn on the IDG. The frequency meter should read approximately 10-11 kHz. Adjust the frequency to 10.2 kHz and lock it by placing the latch switch in the up position. The "A/D latched" LED should change from red to green.

14. Turn on the stroboscope. After a brief warm-up period, the lamp should begin to flash. Change the "Strobe Sync" to 1000. The lamp should flash 10 times more slowly. Return the strobe sync to 100. Change the Divider B Adjust to 20. Again, the lamp should flash 10 times more slowly. Change the strobe sync to 10. The lamp should flash at the

original rate.

15. Change the frequency meter select to "PT in." The meter should read the droplet pulsing frequency. This should be the bimorph driver frequency divided by 1 plus the number on the thumbwheel.

16. Connect the output labeled "TO BIM" on the IDG to the bimorph clamp. The yellow wire (ground) attaches to the thumbscrew on the back of the Plexiglas. The driver signal (clear insulation) attaches to screw on the clamp situated opposite the knurled adjustment screw.

17. Clamp the bimorph between the rear of the brass block and the non-insulated leg of the clamp.

18. Turn the IDG back on and slowly increase the bimorph amplitude. With a frequency of 10.2 kHz, a clear tone should be heard from the bimorph.

19. Connect the pulsing electrode to the red banana jack labeled "TO PULS ELEC." Turn the instrument back on and slowly increase the pulse amplitude. The meter should appear steady. An oscilloscope can be connected to the pulsing electrode to observe the waveform.

20. The instrument should now be ready to produce droplets in the stand-alone mode according to the procedure given in the applications chapter of this dissertation. If any of the above tests did not work, refer to the troubleshooting section below.

B. Production of Neutral Droplets

To produce neutral droplets (i.e., charge the rest of the stream) make the following addition to the above procedure.

1. Connect the "Sync Out B" to the "Ext In Bimorph Driver" on the IDG.

2. Change the "Pulse Trigger Source" switch to the external position (down).

C. Use of the 10 MHz Oscillator

The CTFM contains a 10 MHz oscillator which can be used as an alternate input source to divider A. This oscillator offers the advantage of having much higher resolution than the internal 1 MHz oscillator. However, care must be taken in its use, as it is possible to input too high a frequency to the bimorph driver. Use the following procedure.

1. Connect the yellow banana jack on the CTFM (AUX) to the "Ext In Div A" input on the IDG.

2. Place the bimorph driver source switch in the external (down) position.

3. With the divider A source switch in the internal position, and the frequency selector switch on "A out" adjust the frequency to a value less than 5 kHz.

4. Change the divider A source switch to the external position. The frequency reading should increase by a factor of 10.

5. Adjust the frequency to the exact value desired (do not exceed 50 kHz for long periods). Much better resolution should be available than with the internal 1 MHz oscillator.

6. Return the bimorph driver source switch to the internal position.

7. Return the frequency meter select switch to the bimorph driver in position.

D. Operation in the Computer-controlled Mode

To operate the IDG in the computer-controlled mode, first follow the procedure for the stand-alone mode. If everything appears to be working properly perform these additional steps:

1. Connect the "AM9513-A counter 1" output on the back of the micro to the bimorph driver external input. Be careful not to accidentally connect it to the bimorph driver output! With a T-connector, also connect it to the divider B external input.

2. Connect the "AM9513-B counter 3" output on the back of the micro to the pulse trigger external input on the IDG. Also connect the micro digital ground (the DAC ground is suitable) to one of the IDG BNC grounds.

3. Log in to the HPLC account and load block 9. If unsure of how to do this consult the procedure outlined in Appendix B.

4. Type IDG and return to load the IDG software.

5. Test the software by saying 1 2 10.20 IDG. The program should respond by showing the two closest frequencies and the one actually used. These parameters are the same as those used in the stand-alone procedure above. The 1 represents 1 droplet charged, and the 2 represents two neutral drops.

6. Place the bimorph driver source toggle to external.

7. Place the pulsing trigger source toggle to external.

8. Place the divider B source toggle to external.

9. Change the frequency meter select switch to bimorph driver in. The frequency should be 10.2 kHz.

10. Change the frequency meter select switch to pulse trigger in. The frequency should be 3.55 kHz.

11. To view the droplets produced in this manner, the flashing frequency must be manually adjusted by varying the divider B adjustment thumbwheel. In this case a value of 002 should stabilize the stream.

E. Grounding Considerations

Several ground connections are sometimes needed to stabilize the droplet stream. The liquid delivery vessel should be grounded to assist in droplet charging. If a ringstand is used to hold the bimorph mount, the ringstand should also be grounded to prevent 60 Hz pickup. Pickup of 60 Hz noise makes the droplet stream appear to travel in a sinusoidal trajectory.

If a trap is used to catch charged droplets, the trap should be grounded or else charge will build up on the trap and alter the droplet trajectory. Likewise, any nearby object which can pick up static charges or act as an antenna should be grounded. The IDG electronics case or high voltage case can be used as ground points.

F. Troubleshooting

Symptom: Frequency meter noisy and gives incorrect frequency.

Correction:

1. Decrease bimorph driver amplitude when measuring frequency.

2. Use frequency meter select on bimorph driver input instead of divider A output.

Symptom: Frequency meter reads abnormally high frequencies from divider A.

Cause/Correction:

1. Instrument was turned off and on too fast with divider A latched. The instrument must be allowed to sit for approximately 3 minutes after being turned off before being turned back on, or divider A must be in the unlatched mode.

2. External 10 MHz oscillator being used. Adjust divider A to lower frequency to less than 50 kHz.

Symptom: No output from bimorph driver.

Cause/Correction:

1. Bimorph driver source toggle on "external" with no signal connected.

2. Bimorph driver was operated at too high of an input frequency. Check transistors on bimorph driver and replace if necessary.

3. Short circuit in bimorph clamp assembly. Check for continuity.

Symptom: No pulsing voltage.

Cause/Correction:

1. Check transistors on power supply board and replace if necessary.

Symptom: Erratic strobe lamp operation.

Cause/Correction:

1. Lamp being operated at too high a frequency. Reduce frequency.

2. Lamp being operated at too high an intensity. Use lower intensity setting.

Symptom: Cannot charge droplets.

Cause/Correction:

1. Droplet stream too fast. Use slower flow rate.

2. Solvent cannot be charged. Use different solvent or solvent mix with supporting electrolyte.

3. High voltage power supply not turned on.

4. Solution delivery vessel not grounded.

5. Broken cable to pulsing electrode. Check for continuity.

6. Phasing not properly adjusted. Adjust bimorph amplitude in stand-alone mode or use phasing keys in computer controlled mode.

Symptom: Droplet stream will not remain stable for long periods.

Cause/Correction:

1. Flow rate changing. If constant pressure delivery is being used, then the in-line filter may be dirty. Replace filter element.

2. Frequency not latched. Place latch toggle in up position to lock frequency.

3. Marginal production frequency used. Use better frequency.

Suggestions for IDG-LIBS Research

One of the main considerations in isolated droplet introduction to a laser spark is phasing of the laser trigger with droplet introduction. Fortunately, the IDG can generate the trigger signal in either the stand-alone mode or the computer-controlled mode.

In the stand-alone mode, the easiest introduction technique is to put the entire droplet stream through the plasma region and use the second frequency divider (divider B), normally used for pulsing droplets, as a trigger signal for the laser. Phasing is then accomplished by physically moving the bimorph mount closer or farther away from the plasma using a micro-positioner. Most likely a signal translation module would have to be built to convert the TTL-based divider B output to a signal appropriate for the laser trigger.

In the computer-controlled mode, a similar strategy is used. The entire droplet stream is sent through the focal point, and the 9513 counter normally used to trigger the pulsing circuit is used to trigger the laser. Phasing in this case can be accomplished electronically using the phasing keys. Phasing can be adjusted in increments of 1 μs under computer control. A signal translation module would still have to be built, as the 9513 output is a TTL-based signal. A serial line to an 11/23 supporting FORTHPIP would also have to be available.