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SUBJECT: Elution Electrophoresis as a Clinical Tool

Authors: G.R. Wilkes, R.J. Bertolami, W.M. Choi, and P.D. Terpandjian

Consultant: R.K. Genung

ABSTRACT

An experimental investigation of an elution electrophoresis system with a particulate gel packed column was performed. The operating parameters of the apparatus were determined with elution flow studies and electrophoresis migration studies. Flow study results indicated that sample dispersion is dependent on packing type, packing size, elution flow rate, and outlet geometry. Migration studies revealed that sample migration is dependent upon the packing type, packing size, buffer solution, and applied voltage. The best available operating system was selected from a composite of the results. Minimum criteria for feasibility were an established difference in migration rates for different pure samples and maintenance of the separation during elution.

Electrophoresis at 1000 v in a 40-cm column packed with Bio-Gel P2, 200-400-mesh packing was performed on a sample for 30 min. The sample was eluted from the column at an eluent flow rate of 8 ml/hr. Measurable migration rates were obtained for an albumin sample. Migration for pure samples of A, AC, and F hemoglobins was not apparent. Also, electrophoresis of a mixture of these hemoglobins at the operating conditions optimum for albumin migration produced no separation of the hemoglobin mixture. Lack of migration and lack of separation with the hemoglobin samples led to the conclusion that the present operating conditions and apparatus are unsuitable for analysis of hemoglobins. A new column design for future experimentation is suggested.

Oak Ridge Station
School of Chemical Engineering Practice
Massachusetts Institute of Technology

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Contents

1. SUMMARY

Elution electrophoresis has been proposed as a technique for clinical analysis of biological macromolecules (1). In this technique, electrophoresis of a sample in a packed column for a period of time is followed by elution from the column and protein detection by ultraviolet spectroscopy. An experimental investigation of this method was performed with a 40-cm horizontal column packed with Bio-Gel particulate gel packing. The goal of the project was to establish the nature and effects of the operating parameters and to develop a successful operating system for the analysis of a serum protein sample. The criteria for the establishment of a successful operating system was the demonstration of different component protein migration rates in electrophoresis and maintenance of the achieved separation during elution.

Elution studies determined the relation between dispersion of the sample and the eluent flow rate in different packings. Polyacrylamide Bio-Gel P2 packings of 100-200, 200-400, and -400 mesh, and polyacrylamide with 6% agarose Bio-Gel A0.5m packings of 100-200 and 200-400 mesh size were used for the elution flow studies. For the 10-cm column, only the P-series packings were examined before the column was broken. Results of the studies in the 10-cm column indicated that dispersion was smallest with the 200-400 mesh size packing. Dispersion was also found to decrease as a function of increasing flow rate. In the 40-cm column, all packings were examined. The smallest sample dispersion was observed with the 100-200 mesh A-series packing. Experimental problems of packing compaction and clogging developed with the -400 mesh P2 and the 200-400 mesh A0.5m packings. The dispersion of the sample in the other three packings was observed to increase with flow rate. Minimum dispersion with the 200-400 mesh P2 packing led to the speculation that the 200-400 mesh A0.5m packing should produce the minimum sample dispersion of all of the packings examined. Analysis of the concentration-time curves obtained during the elution studies led to the conclusion that the dispersion of the sample in the 40-cm column could be reasonably fit with a closed vessel dispersion model. The dispersion in this column was small. Analysis of the similar curves for the 10-cm column indicated that the large amounts of dispersion could be attributed to a stagnant flow region near the eluent outlet.

The electrophoretic migration studies examined the parameters of packing type, packing size, applied voltage, and buffer solution in the 40-cm column with an albumin sample. The five different packings examined in the flow studies were also available for the migration studies; however, the -400 mesh P2 packing was not examined and the 200-400 mesh A0.5m packing produced questionable results. Applied voltages in the range of 500-1250 v in multiples of 250 v were used to establish the electrophoretic mobilities. Two different buffer solutions were examined: a 0.065 M tris (hydroxymethyl) aminomethane and 0.018 M boric acid buffer, and a 0.25 M dibasic sodium phosphate buffer. The goal of the migration studies was to determine the packing in which an albumin sample demonstrated the greatest migration rate. The results of the packing size comparison indicated that the 200-400 mesh size was the best packing size. The results of the comparison of packing

type again indicated that A-series packing was the better packing. Thus, from the composite of these comparisons, it was concluded that the 200-400 A-series packing was the best available packing; however, once again, as with the flow studies, the operation of the apparatus with this packing was seriously hampered by packing compaction and clogging.

From the migration studies and the flow studies, an operating system was selected with the goal of maximizing mobility for an albumin sample in electrophoresis and of minimizing the sample dispersion during elution. The system parameters selected for analysis of the serum proteins and the hemoglobin samples were electrophoresis at 1000 v in the 40-cm column packed with 200-400 mesh P2 packing for 30 min followed by immediate elution at an eluent flow rate of 8 ml/hr. A 3-μl sample of albumin showed a migration rate of 0.32 cm/min under these operating conditions.

Three pure hemoglobin samples, A, AC, and F, were analyzed individually, and a mixture of the three samples analyzed with the selected operating conditions. Results of the analysis did not indicate any migration for any of the hemoglobins under the same operating conditions in which albumins demonstrated a mobility of 0.32 cm/min. A sample of blood serum with at least 29 known protein components was analyzed with the same operating system. Recorded results of the serum sample analysis indicated that no separation of the components of the serum was obtained. Since the criterion for feasibility of the elution electrophoresis apparatus was the establishment of different protein mobilities in a mixed protein sample, then it must be concluded based on the above results with the serum and the hemoglobin samples, that the present experimental operating conditions and apparatus are not suitable for this application.

2. INTRODUCTION

2.1 Clinical Value of Elution Electrophoresis

Gel electrophoresis and cellulose strip electrophoresis are the two primary techniques for clinical analysis of blood serums and similar mixtures of complex biological macromolecules. Of the two processes, gel electrophoresis is the method of analysis providing the highest resolution. The technique of gel electrophoresis, however, requires that a series of complicated, time-consuming steps be executed by a skilled laboratory technician. The most difficult operation is casting the gel which requires twenty-four hours. The clinical method substitutes pre-cut cellulose strips for the gel as the stationary media for separation. The advantage of the cellulose strip method is the removal of the complexity of gel casting; however, the use of the strips yields diminished resolution over the gel.

A system of automated elution electrophoresis would offer advantages over both of the other methods of clinical analysis (1) if a successfully operating system could be developed. The advantage of automated elution electrophoresis over gel electrophoresis is the speed of operation; an

automated method providing 30 min of electrophoresis followed by elution at normally employed flow rates could perform one analysis per hour as opposed to the 24 hr required of gel electrophoresis. Furthermore, such a system would not require the full-time attention of a skilled laboratory technician for operation.

2.2 Background

Electrophoresis is the transport of electrically charged particles in a direct current electrical field. These particles may be simple ions, complex macromolecules, colloids, living cells, or inert materials, such as oil emulsion droplets (2). Electrophoresis can be applied to separate charged components of different mobilities in liquid solution. The mobility of a particle is the rate at which the particle migrates under the influence of an electrical field and is affected by electrical properties and viscosity of the surrounding environment.

Elution is the process of washing a protein sample from the packing material by the forced flow of a solution. The potential of combining elution and electrophoresis into one process, elution electrophoresis, for use as a clinical tool has been demonstrated (1). Retainment of component separation achieved in electrophoresis is a necessary condition of the elution step for high resolution required by analysis. Several undesirable effects, such as dispersion of the sample may occur during the elution process. Sample denaturation may also occur if there is chemical reaction with the packing material.

Electrophoresis as an analytical tool was first demonstrated by Tiselius in 1937 (2). Analytical separation techniques for characterizations of complex biological mixtures have been developed with the electrophoresis method of Tiselius. These processes can be conducted either without supporting media, free electrophoresis, or with supporting media, zone electrophoresis. The purpose of the supporting medium is to act as a barrier against convection, thereby stabilizing the separated zones. At least six techniques of free electrophoresis and many different techniques of zone electrophoresis have been established (2).

Free electrophoresis techniques are employed primarily for preparative purposes in the production of protein samples rather than analytical methods because of diminished resolution caused by diffusion. Zone electrophoresis has been employed for both analytical and preparative purposes. Gel electrophoresis is a zone electrophoresis technique with a gel material, such as agar or starch, as the supporting media. Detailed studies of gel electrophoresis have been made using both agar and starch gels (3, 4, 5).

Several theories for free electrophoresis have been proposed; however, the application of free electrophoresis theory to protein electrophoresis has not proven satisfactory (2). Similar treatments for the theory of zone electrophoresis have not yet been established. Free electrophoresis theory predicts that the parameters affecting migration rate are the surface charge

distribution on the particle, the size of the particle, the electrical field strength in the vicinity of the particle, the mobilities, charge distribution, and concentration of the ions of the liquid surrounding the particles, and the physical properties of the liquid, such as viscosity and dielectric constant. Theory of zone electrophoresis further predicts that the effects of packing distribution and relative ionic, protein, and packing sizes are important parameters. A simple model of the effects of packing has been suggested by Kunkel and Trautman (6).

2.3 Objectives

The objectives are to develop a working experimental system for elution electrophoresis in particulate gel supporting medium and to establish the operating conditions required for clinical analysis of serum proteins and similar biological mixtures.

2.4 Method of Attack

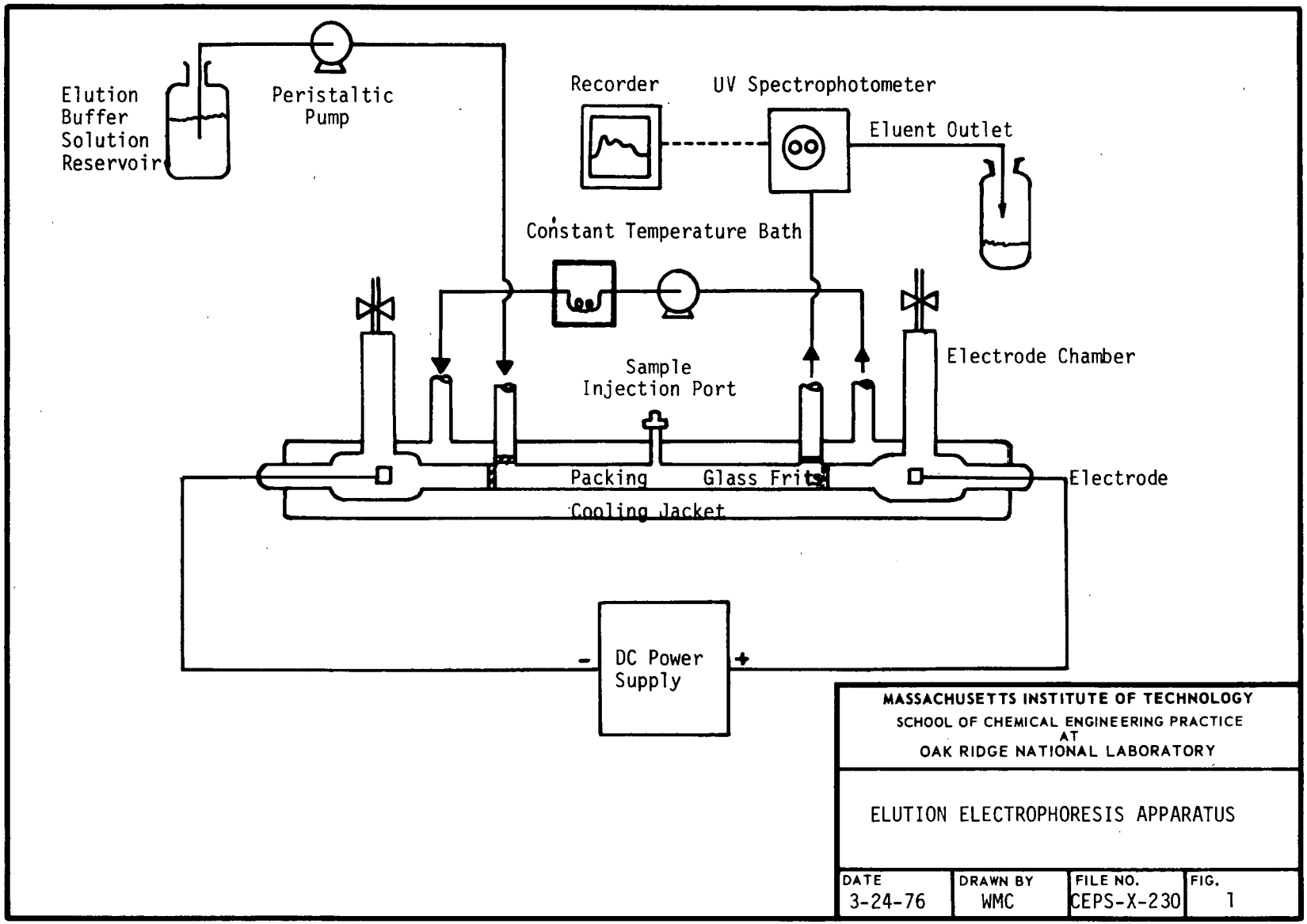
Preliminary work included both flow studies and electrical resistance measurements. The purpose of the flow studies was to determine experimentally the most favorable operating parameters for sample elution with the existing apparatus by examining the effects of parameters that could be readily varied. These parameters were elution flow rate, packing size, packing type, and column geometry. The electrical measurements were performed to establish the effects on the applied voltage gradient caused by buffer solution type and the column construction required to constrain the packing media.

In the migration studies the parameters of surrounding liquid composition, applied voltage,and packing size and type on the electrophoretic operation of the apparatus were investigated. Migration rates of two different protein samples were to be established at several applied voltages in different packings. Criteria for a feasible system of elution electrophoresis are the demonstration of different electrophoretic migration rates for these two different protein samples and maintenance of this separation during elution.

3. APPARATUS AND PROCEDURE

3.1 Apparatus

A schematic diagram of the experimental apparatus is presented in Fig. 1. A 48-cm column of 4.3-mm-ID glass tubing equipped with a cooling jacket is mounted inside an acrylic plastic housing. The housing is equipped with safety switches to prevent sample introduction or other system changes while



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ELUTION ELECTROPHORESIS APPARATUS

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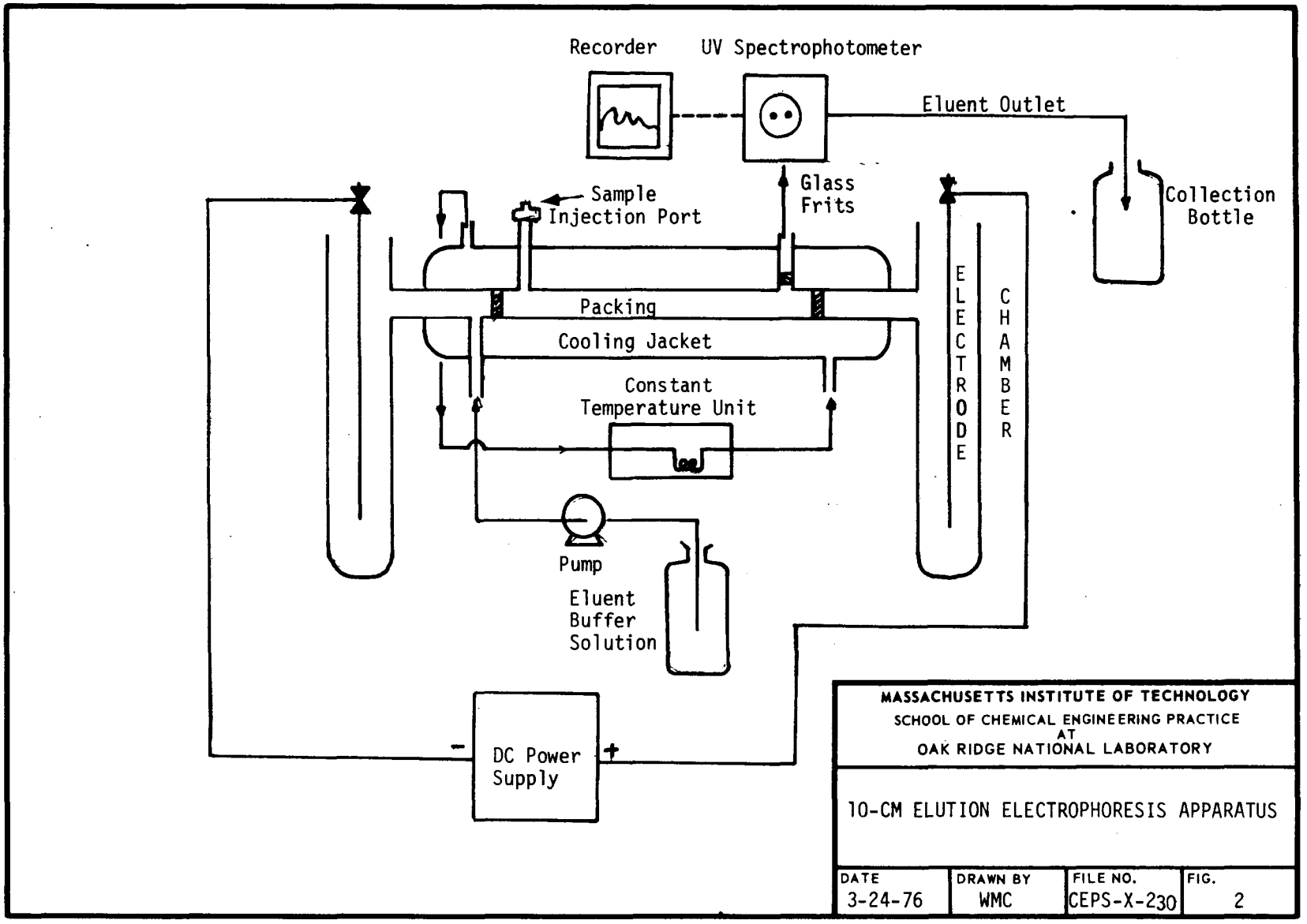
the high voltage power supply, connected to two electrodes at opposite ends of the apparatus, is in operation. The column is provided with two glass frits, 2-mm thick, placed 40 cm apart to secure the column packing. The frits are permeable to the flow of buffer solution. Vents are supplied at each electrode chamber for removal of electrolysis gases produced during electrophoresis. A sample injection port is located at the midpoint between the two frits. Two glass tubes are placed in the column as shown for inlet and outlet of the eluent solution. Both of these tubes are provided with glass frits and pass through the outer wall of the cooling jacket. The eluent inlet tube is connected by plastic capillary tubing to the outlet of a peristaltic pump. The inlet to this pump is connected as shown to a bottle containing buffer solution. The outlet eluent tube is connected by capillary tubing to an ultraviolet flow spectrophotometer set at 280 nm. The output signal of the spectrophotometer is recorded on a strip chart recorder. The eluent solution passes through the spectrophotometer and is collected in a bottle for flow rate or pH measurements. Water at 0°C is circulated from the constant temperature bath through the cooling jacket. The column is designed to allow electrophoresis to proceed with or without simultaneous elution. The present electrophoresis experiments were performed without simultaneous elution.

Two types of Bio-Gel packing were available, Series P2 packing - spherical particles made of polyacrylamide - and Series A0.5m packing - spherical particles made of polyacrylamide in a powder of 6% agarose. The P2 packing was available in three sizes: 100-200 mesh, 200-400 mesh, and -400 mesh. The A0.5m packing was available in the 100-200 mesh and 200-400 mesh sizes. The 100-200 mesh and 200-400 particles have a distribution of particle diameters in the range of 74 to 149 and 37-74 μm, respectively. The -400 mesh particles have diameters less than 37 μm.

Two different buffer solutions were employed. The tris-borate buffer was a 0.065 M aqueous solution of tris(hydroxymethyl)aminomethane and 0.018 M boric acid. The phosphate buffer was a solution of 0.25 M dibasic sodium phosphate. Both solutions were protected from bacterial attack by trace amounts of sodium azide. The pH of both buffers was 8.9.

For the preliminary investigations of the flow studies, additional 10-cm glass columns equipped with cooling jackets and constructed as shown in Fig. 2 were available. Columns of this construction would be used for analysis of protein samples in which all components of the protein have the same sign of charge when in the environment of the buffer solution. One of these columns had glass frits for the packing support; the other had no prefabricated supports for the packing. The 10-cm columns were not equipped with prefabricated electrode chambers, thus standard 6-in. test tubes equipped with side arms were connected to the column with tygon tubing as electrode chambers. The packing in the column without the glass frits was secured by a glass wool stopper. The sample injection port for these columns was located at the end of the column.


Recorder
UV Spectrophotometer
Eluent Outlet
Collection Bottle
Sample Injection Port
Glass Frits
Packing
Cooling Jacket
Constant Temperature Unit
ELECTRODE CHAMBER
Pump
Eluent Buffer Solution
DC Power Supply
-
+
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10-CM ELUTION ELECTROPHORESIS APPARATUS
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FIG. 2

3.2 Procedure

To pack the column, the lines connecting the eluent pump to the eluent inlet port and the eluent outlet port to the UV spectrophotometer are disconnected. This allows the buffer solution, originally occupying the column, to be discharged when packing begins. The system is removed from the sample injection port and is replaced by a piece of tygon tubing approximately 10 cm in length and 1 cm in diameter. The tygon tubing is filled with packing. The eluent pump is connected to the tubing. The flow of buffer solution from the eluent pump forces the packing into the column. When the column is totally packed, the septum is replaced. The lines connecting the eluent pump to the eluent inlet port and the eluent outlet port to the UV spectrophotometer are reconnected.

After packing, the column was flushed and filled with buffer solution. The electrodes were connected to the power supply, and the electrode chamber vents were opened for removal of the gas which evolves during electrophoresis. A 3-$\mu\ell$ protein sample was introduced through the sample injection port. Direct current potential differences in the range of 300-1200 v were applied to the electrodes to begin the electrophoresis. To establish the migration rate of the sample, the voltage was applied for a period of 30 min. After the power supply has been turned off and the vents on the electrode chambers were closed, the sample was eluted from the column with buffer solution flow rates of $\sim$8 ml/hr. The eluent stream is monitored continuously by the spectrophotometer. Results were taken from the strip chart recording and analyzed. During the migration studies, the migration rate was calculated from the strip chart (as shown in Appendix 10.1) for several voltages and column packings. Flow studies were performed without electrophoresis for several flow rates in the range 0-15 ml/hr.

4. THEORETICAL APPROACH

4.1 Free Electrophoresis Theory

Theories of free electrophoresis have been developed (7); however, all of these theories are derived with the basic assumption of a large colloidal particle and are applicable only to colloids. Extension of these theories to protein solutions has been very unsatisfactory (8). Further extension to zone electrophoresis has been only qualitative (6).

Free electrophoretic mobilities in terms of measurable properties may be calculated from the Henry equation. More elaborate equations, such as those of Booth and of Overbeek reduce to the Henry equation as a limiting case. This equation (8) is

$$U = \frac{v}{E} = \frac{Ze}{6\pi\mu R} \frac{X_1(\kappa R)}{1 + \kappa R} \tag{1}$$

where $X_1(\kappa R)$ is Henry's function given by

$$X_1(\kappa R) = 1 + \frac{1}{16}\kappa^2R^2 - \frac{5}{48}\kappa^3R^3 - \frac{1}{96}\kappa^4R^4 + \frac{5}{96}\kappa^5R^5 + \frac{11}{96}e^{\kappa R}\int_{-\infty}^{\kappa R}\frac{e^{-\ell}}{\ell}d\ell \tag{2}$$

and where the Debye length, κ, is

$$\kappa = \sqrt{\frac{4\pi e \sum_i c_i Z_i^2}{\epsilon kT}} \tag{3}$$

A brief derivation emphasizing the assumptions of Eq. (1) is presented here. A more mathematical treatment is given by Henry (9).

A rigid, spherical particle is placed in an electrolyte solution in which there is only one species of cation and anion present. The charge on the particle is uniformly distributed on the surface of the sphere. An electric field exists throughout the liquid medium. For a particle moving at steady state in one dimension in an electrical field, the forces on the particle are as shown in Fig. 3.

At steady state, Newton's Law requires

$$\sum_i F_i = 0 \tag{4}$$

The forces acting on the particle are friction, static electrical forces, and electrical forces due to interaction of moving charged particles. Thus Eq. (4) becomes

$$F_e + F_f + F_r + F_v = 0 \tag{5}$$

where F_e is the coulombic force in the electrical field given by

$$F_e = Z_i e E \tag{6}$$

F_f is the frictional force given by Stokes' Law.

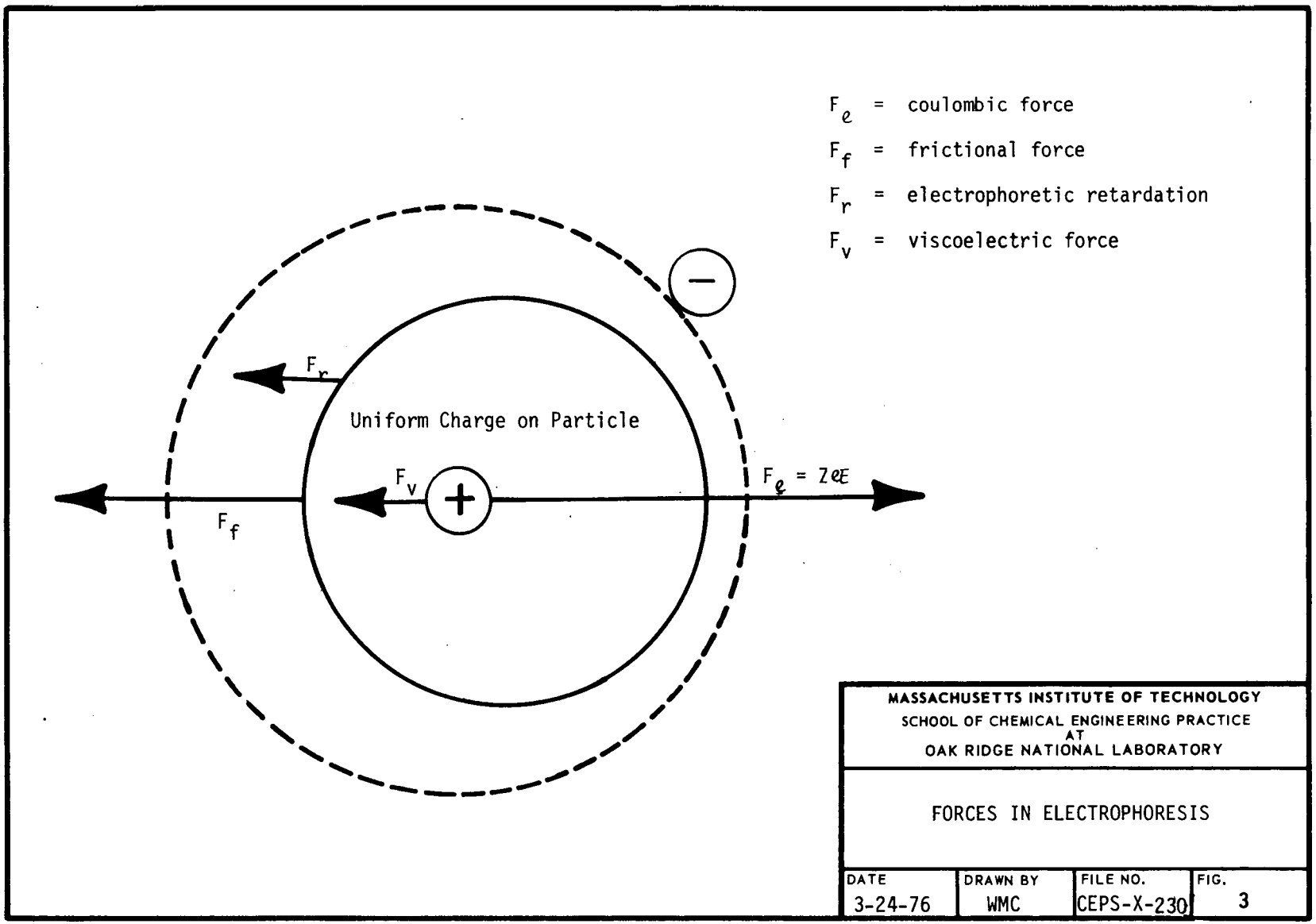
F_e = coulombic force
F_f = frictional force
F_r = electrophoretic retardation
F_v = viscoelectric force
F_r
Uniform Charge on Particle
F_v
$F_e = ZeE$
F_f
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FIG. 3

$$F_f = -6\pi\mu Rv \tag{7}$$

F_r and F_v are forces due to electrophoretic retardation and viscoelectric effects, respectively. The form of the equation to express these forces is determined by the assumptions necessary to describe the double layer of charge around the particle (10). The so-called double layer is caused by polarization of the freely moving ions of the surrounding solution. Conceptually, these equations may be expressed as

$$F_r = -f_1 \text{ (local electric field, particle dimensions, ionic charges, ionic concentrations, ionic mobilities)} \tag{8}$$

and

$$F_v = -f_2 \text{ (local electric field, particle dimensions, ionic charges, ionic concentrations, ionic mobilities)} \tag{9}$$

The electrophoretic retardation occurs because of the existence of the double layer; the viscoelectric forces arise from rearrangement of the double layer of charge as the particle migrates. Equations (5) through (9) may be rearranged to the form

$$U = \frac{v}{E} = \frac{Ze}{6\pi\mu R}\left(1 - \frac{f_1}{ZeE} - \frac{f_2}{ZeE}\right) \tag{10}$$

As viscoelectric effects are neglected in derivation, f_2 is equal to zero. The magnitude of the electrophoretic retardation force is comparable to the coulombic and frictional forces. The form of the electrophoretic retardation equation, f_1, in Henry's derivation gives Eq. (1) from Eq. (10). More elaborate equations are presented by Overbeek and Wiersma (7).

4.2 Dispersion Theory

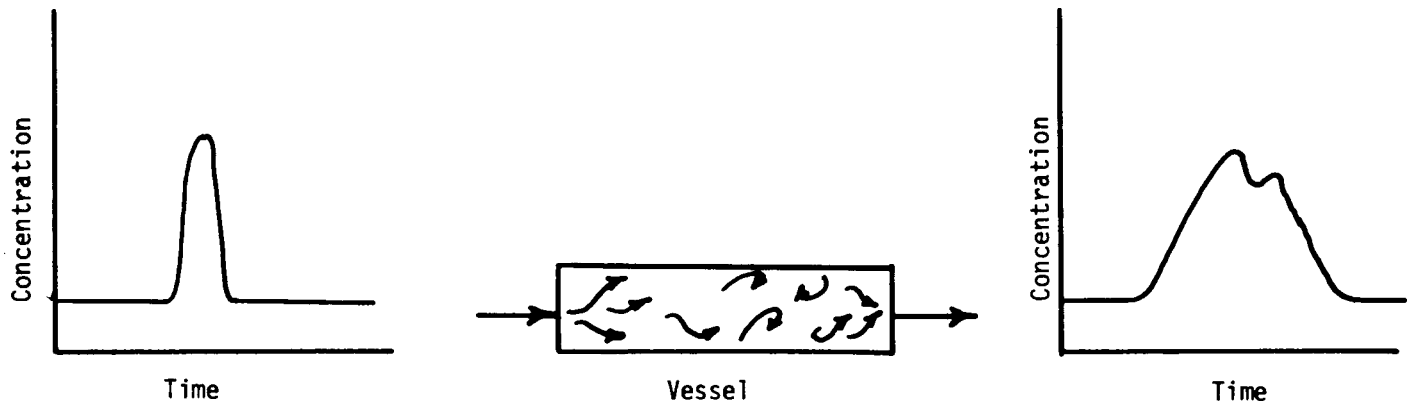
The model of dispersed plug flow (11) can be employed to interpret the UV spectrophotometer response during elution. This dispersion model predicts that the concentration profile in time at a fixed point in the system of interest is related to the deviation from plug flow behavior. Plug flow and well-mixed flow are two idealized flow patterns. Plug flow corresponds to the limit of zero dispersion. Well-mixed flow corresponds to the limit of infinite dispersion. All real systems will have some measurable dispersion.

The distribution of residence times required for a differential volume element of fluid to pass through a vessel is determined by the path that each element must take. The path of a fluid element varies depending upon the exact shape of the flow areas in the vessel. The residence time distribution is thus broadened if some fluid volume elements may move much more quickly than the bulk of the flow, such as in channeling; or much more slowly, such as in a stagnant region. Channeling occurs when a path that is a shorter route for an element of the entire flow develops in the vessel. Stagnation is generally caused by fluid recirculation in some sections of the vessel. Both channeling and stagnation will tend to broaden a residence time distribution.

Dispersion is generally measured with recording devices that are sensitive to the concentration of some "tracer" component in the fluid. A tracer component must be detectable and yet must not disturb the flow. A typical recording device is one that measures the absorption of light at a known wavelength by a tracer. The required concentration of the tracer is determined by the sensitivity of the detection device.

To obtain information about the flow patterns, the sample must be introduced into the system in some known manner such as periodic signal, or step, or pulse stimulus. For a pulse stimulus corresponding to the mathematical pulse or δ-function, typical response curves for a closed vessel are shown in Fig. 4. It is important to distinguish between two different types of vessels. A closed vessel is a region bounded by areas of zero dispersion. An open vessel has uniform dispersion everywhere. This is shown conceptually in Fig. 5. A closed vessel is one in which both the sample injection and the response detection take place outside of the region of finite dispersion. The sample enters and leaves the vessel by plug flow. An open vessel corresponds to the opposite case.

Assume that a tracer is injected into a uniform plug of essentially zero thickness. The concentration in each element of fluid contained in the plug is the same as every other element. As the plug of tracer enters the bed, each fluid element must flow around the packing in its path. The path of each fluid element in the plug cannot be the same, thus different path lengths result as shown in Fig. 6. Eventually all fluid elements entering the bed must come out of the bed. The residence time distribution of the fluid elements in the vessel is a measure of the dispersion in the vessel. Levenspiel presents curves and models such as those in Fig. 7 for calculation of dispersion based on the closed vessel analysis. Application of these models to the present elution electrophoresis system is presented in Appendix 10.1.3. Data may be compared to the reference curves for quantitative and qualitative measures of dispersion.



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TYPICAL STIMULUS RESPONSE CURVES			
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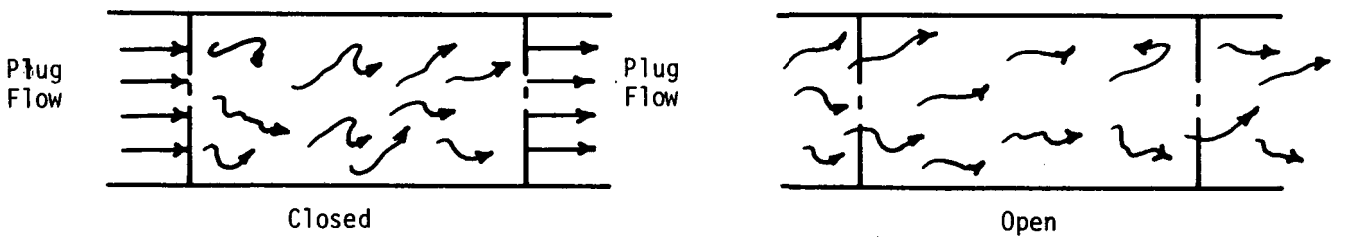

MASSACHUSETTS INSTITUTE OF TECHNOLOGY SCHOOL OF CHEMICAL ENGINEERING PRACTICE AT OAK RIDGE NATIONAL LABORATORY			
CLOSED AND OPEN FLOW VESSELS			
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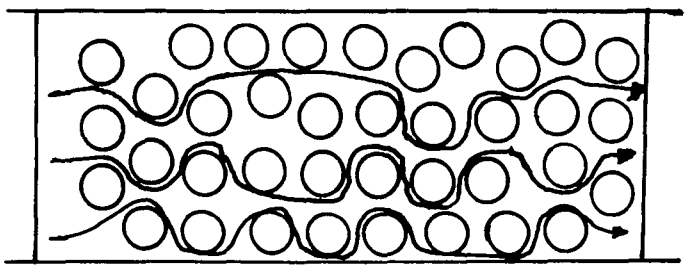

Reactor Volume

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FLOW PATHS IN PACKED COLUMN

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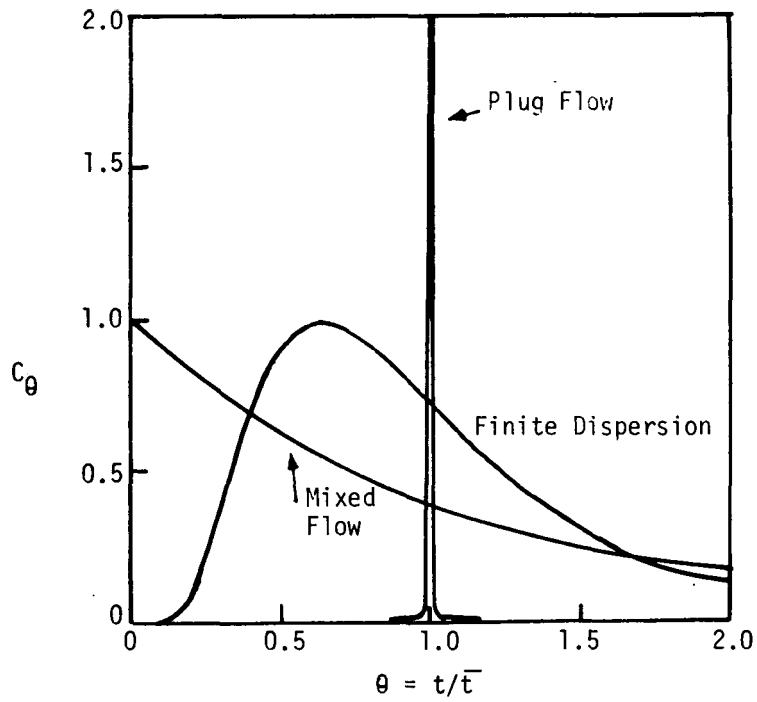


MASSACHUSETTS INSTITUTE OF TECHNOLOGY SCHOOL OF CHEMICAL ENGINEERING PRACTICE AT OAK RIDGE NATIONAL LABORATORY			
C-CURVES IN CLOSED VESSELS FOR VARIOUS EXTENTS OF DISPERSION			
DATE 3-21-76	DRAWN BY RJB	FILE NO. CEPS-X-230	FIG. 7

5. RESULTS

5.1 Serum Analysis

A serum protein sample potentially containing 29 identifiable components was injected into the 40-cm column for analysis. The column was packed with Bio-Gel 200-400 mesh P2 packing with phosphate buffer solution. A dc voltage of 1000 v was applied to the electrodes and maintained for 30 min. The sample was eluted at a flow rate of 6 ml/hr. The serum sample migrated a calculated distance of 5.1 cm during electrophoresis; however, only one peak was observed on the strip chart recorder.

5.2 Hemoglobin Mixture Analysis

Three pure hemoglobin samples, Hemoglobin A, Hemoglobin AC, and Hemoglobin F, were injected into the apparatus, maintained 30 min at 1000 v, and eluted with approximately an 8 ml/hr elution flow rate. Migration was not observed for any of the three hemoglobin samples. In these experiments with the individual samples, the migration could also be observed visually because the samples were red in color and could be seen after injection.

A mixture of the three hemoglobins was injected into the column for analysis under the same conditions. During the electrophoresis period, migration was not apparent in visual observations. The subsequent elution at 8 ml/hr produced only one peak on the recorder output.

5.3 Migration Experiments

Time limited the experimental work to a detailed examination of electrophoresis with only one protein sample, albumin, and only one period of applied voltage, 30 min. The only operating parameters that could be examined in some detail were applied voltage, two buffer solutions, packing size, and packing type. Additionally, the effect of column geometry with the two column sizes was examined to a lesser degree. Experiments with the 10-cm column were terminated when the column was broken.

5.3.1 Effect of Applied Voltage

Electrophoretic mobility of an albumin sample was examined as a function of applied voltage in the 40-cm column for the different packings. The migration rate was calculated from the recorder output, as described in the sample calculation of Appendix 10.1.1. Measurable migration rates were obtained from experiments with only two different packings. A plot of migration rate as a function of applied voltage is presented in Fig. 8 for 200-400 mesh P2 packing and for 100-200 mesh A0.5m packing.

In experiments with the 200-400 mesh P2 packing, three applied voltages, 500, 750, and 1000 v, provided measurable migration rates. Migration was not apparent with an applied voltage of 250 v. A voltage of 1250 v produced electrical arcing throughout the packing and in the electrode chambers. Thus the experiment was aborted. Note that there are two data points in Fig. 8 at an applied voltage of 1000 v.

The observed migration rates shown in Fig. 8 are also plotted against the measured current in Fig. 9 with current as the independent variable instead of voltage. Note that Fig. 9 appears to indicate a linear relationship between the calculated migration rate and measured current.

5.3.2 Effect of Buffer Solution

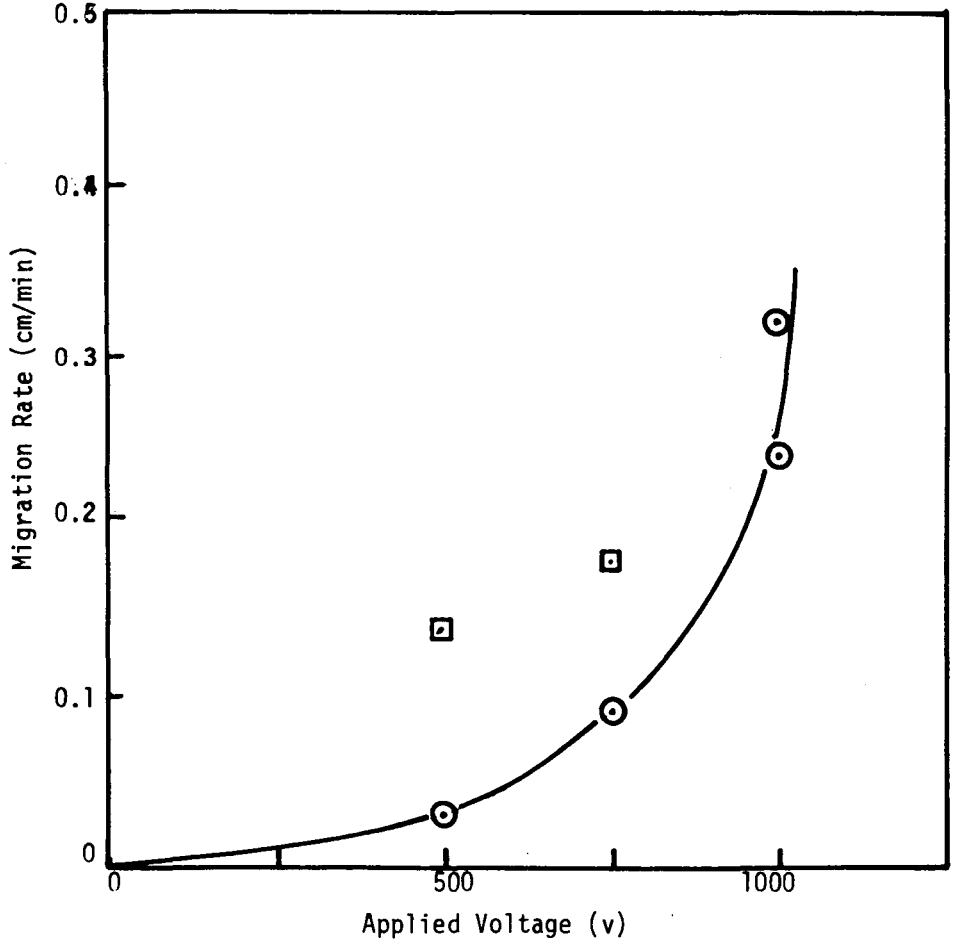
Migration rates with both buffer solutions were calculated from experimental data taken in the 40-cm column packed with 100-200 mesh A packing for applied voltages 0.5 in. from 500 to 1200 v. The measured migration rates for an albumin sample are presented in Table 1 for both the phosphate and borate buffer solutions. Current measurements were not recorded for these experiments.

Table 1. Albumin Migration Rate in Two Buffer Solutions

	Applied Voltage (volts)	Migration Rate (cm/min)
Borate Buffer		
	1000	~0
	1200	~0
Phosphate Buffer		
	500	0.14
	750	0.18

5.3.3 Effect of Packing Type

To establish the effects of packing type on migration rate, experiments were performed with the 40-cm column packed with both the 100-200 mesh P2 packing and the 100-200 mesh A0.5m packing. The column was filled with phosphate buffer solution, a sample of albumin injection applied, voltage applied in the range of 500-1200 v for 30 min, and the sample eluted at a flow rate of 8 ml/hr. The results of these studies are presented in Table 2. It is important to note that measurable migration was not obtained with the 100-200 mesh P2 packing but was obtained with the 100-200 mesh A0.5m packing.



○ 200-400 Mesh P2 Packing

□ 100-200 Mesh A0.5m Packing

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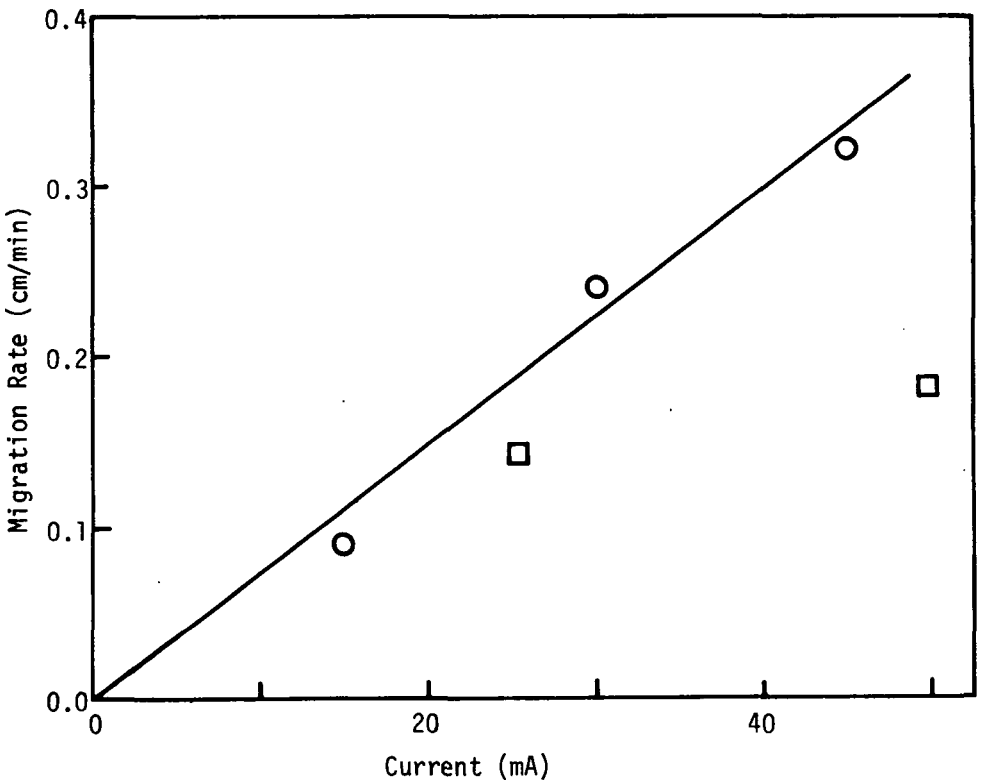
EFFECT OF VOLTAGE ON MIGRATION RATE

DATE	DRAWN BY	FILE NO.	FIG.
3-21-76	WMC	CEPS-X-230	8



○ 200-400 Mesh P2 Packing

□ 100-200 Mesh A0.5m Packing

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EFFECT OF CURRENT ON MIGRATION RATE

DATE	DRAWN BY	FILE NO.	FIG.
3-21-76	WMC	CEPS-X-230	9

Table 2. Albumin Migration Rate with Different Packings

	Applied Voltage	Migration Rate (cm/min)
200-400 Mesh P2 Packing		
	500	0.03
	750	0.09
	1000	0.24
	1000	0.32
100-200 Mesh P2 Packing		
	500	~0
	750	~0
	1000	~0
100-200 Mesh A0.5m Packing		
	500	0.14
	750	0.18

5.3.4 Effect of Packing Size

Experiments were performed to examine the effects of packing size by varying the packing in the 40-cm column while maintaining all other parameters fixed. Results of experiments with 100-200 mesh P2 packing and 200-400 mesh P2 packing are presented in Table 2. Note that migration was observed with the 200-400 mesh P2 packing but not with the 100-200 mesh P2 packing.

Migration experiments were attempted with the 10-cm column using glass wool to secure the packing in place. The preliminary results showed excellent resolution; however, due to equipment failure, the column could not be used for further experiments.

Experiments were also attempted with the 10-cm column with glass frits. Electrical arcing was observed throughout the packing and in the electrode chamber for applied voltages in excess of 360 v. No migration was apparent below 360 v.

5.4 Flow Experiments

Resolution as a function of elution flow rate was investigated in the 10-cm column for 100-200 and 200-400 mesh P2 packing. Resolution was measured by the sample elution volume, the volume of the eluent associated with the total removal of a sample from the packed bed. The results of these experiments are presented in Fig. 10. Similar investigations were attempted using P2 packing of -400 mesh size. However, the glass frits that hold the packing became clogged and caused leaks at many points in the system.

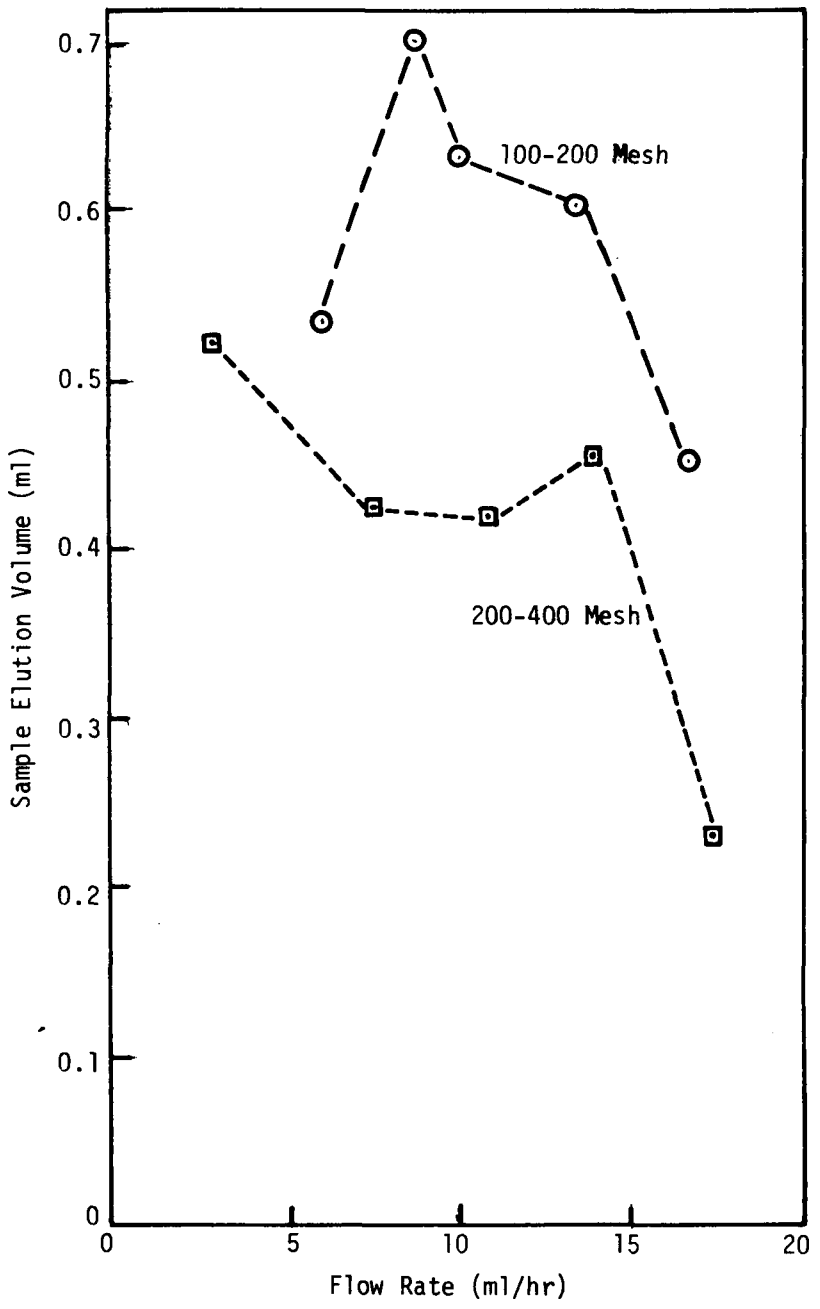
Sample elution volume as a function of elution flow rate was measured for the 40-cm column with 100-200 and 200-400 mesh P2 packing by Genung et al. (12). These results are shown in Fig. 11. The effect of packing type was also measured for 100-200 mesh P2 and A0.5m packings. These results are presented in Fig. 12.

5.5 Resistance Measurements

The resistance of the 10-cm column containing only buffer solution, buffer solution with glass wool, and buffer solution with two glass frits was measured with an ohmmeter. The results presented in Table 3 are the mean values of several measurements with 90% confidence limits.

Table 3. Average Static Resistance Measurements

	Resistance (kΩ)
10-cm Column with Tris Borate Buffer	69 ± 1
10-cm Column with Tris Borate Buffer and Glass Wool	70 ± 1
10-cm Column with Tris Borate Buffer and Glass Frits	110 ± 2
10-cm Column with Phosphate Buffer	19 ± 1
10-cm Column with Phosphate Buffer and Glass Wool	21 ± 1



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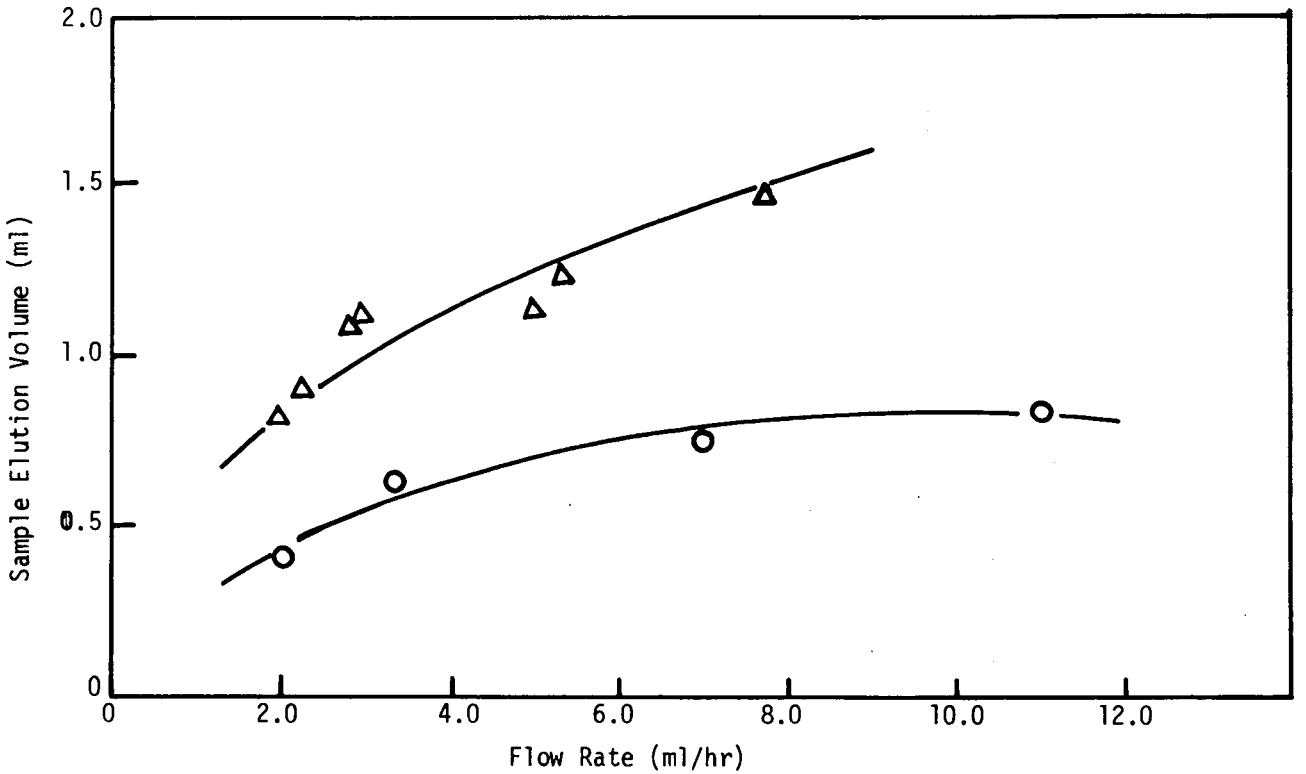
SAMPLE ELUTION VOLUME IN THE 10-CM COLUMN

DATE	DRAWN BY	FILE NO.	FIG.
3-21-76	WMC	CEPS-X-230	10



△ 100-200 P2 Mesh

○ 200-400 P2 Mesh

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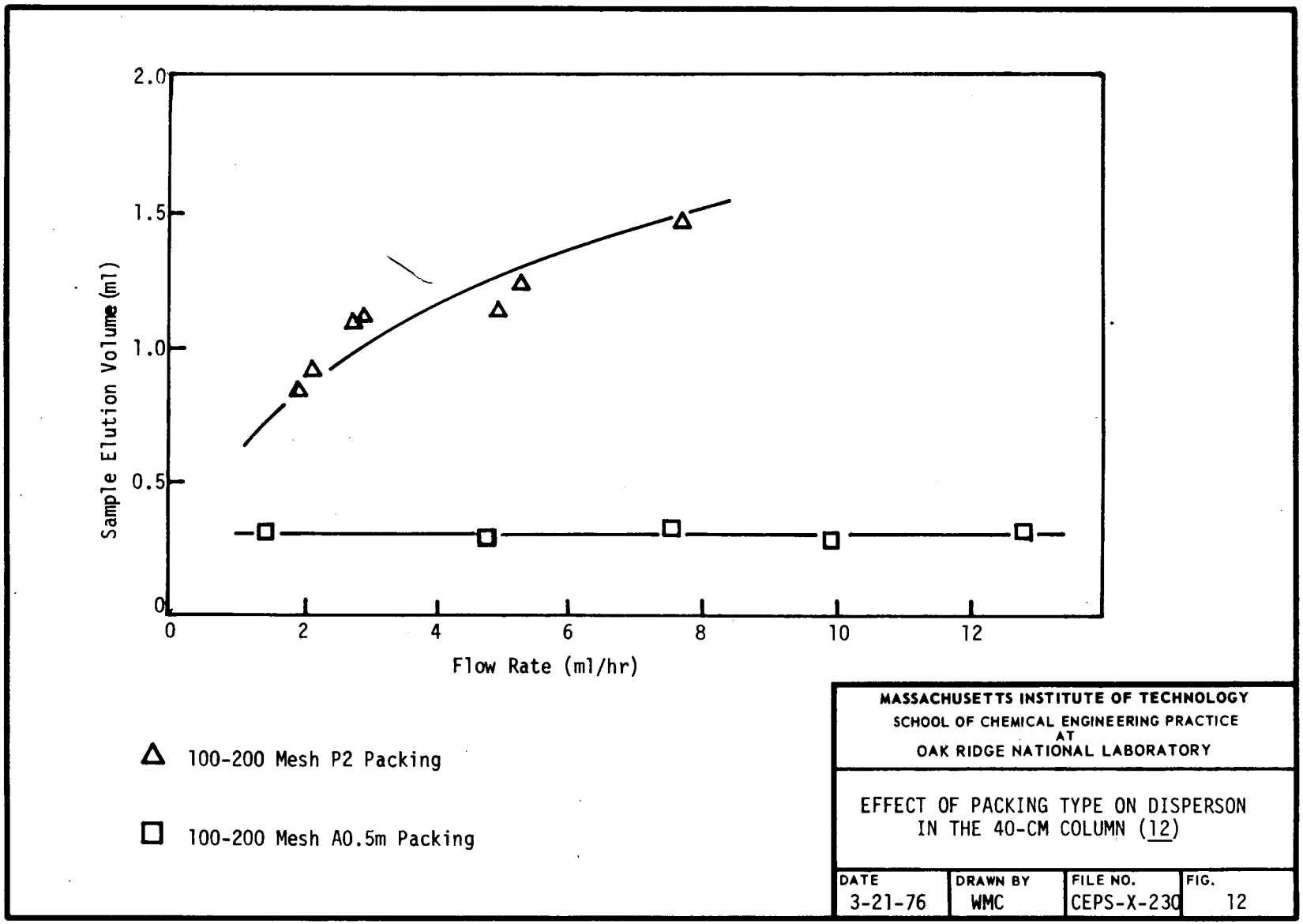
EFFECT OF PACKING SIZE ON DISPERSION IN THE 40-CM COLUMN (12)

DATE	DRAWN BY	FILE NO.	FIG.
3-21-76	WMC	CEPS-X-230	11


2.0
1.5
1.0
0.5
0
Sample Elution Volume (ml)
0
2
4
6
8
10
12
Flow Rate (ml/hr)
100-200 Mesh P2 Packing
100-200 Mesh A0.5m Packing
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EFFECT OF PACKING TYPE ON DISPERSON
IN THE 40-CM COLUMN (12)
DATE
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FILE NO.
CEPS-X-230
FIG.
12

6. DISCUSSION OF RESULTS

6.1 Migration Experiments

6.1.1 Effect of Voltage or Current

Measurable migration of an albumin sample was demonstrated in two packing types, 200-400 mesh P2 and 100-200 mesh A0.5m. In the P2 packing, the migration rate increases slowly with increasing voltage in the range of 0 to 500 v; however, in the range of 500-1000 v, the migration increases more rapidly as shown in Fig. 8. In the 100-200 mesh A0.5m packing, only two data points were obtained. Hence, one may only conclude that migration rate increases with increasing applied voltage and that electrophoresis should be performed at the highest possible voltage for maximum mobility. However, in practice the voltage is limited by the capability to remove heat produced by resistance heating in the packing rather than by the voltage source.

A plot of experimental migration rate as a function of measured current indicates that the migration rate is linearly proportional to current for the P2 packing as shown in Fig. 9. Although only two data points are available for the A-series packing, the relationship does not appear to be linear when the origin is included as a data point.

The results indicate that migration rate increases with increasing current and again indicate application of the highest possible voltage will yield maximum mobility. Since the applied voltage is limited by the cooling capacity of the apparatus, the development of an apparatus with increased cooling capacity is recommended for future investigations.

6.1.2 Effect of Buffer Solution

As shown in Table 1, migration rate was not measurable in the tris borate buffer, but was measurable in the phosphate buffer. As mobility was greater in phosphate than in tris borate buffer, the 0.25 M phosphate buffer was used in preference to the 0.065 M tris borate buffer.

The phosphate and borate buffer had different ionic strengths. The ionic strength of the phosphate buffer (as calculated in Appendix 10.1.4) was 0.75, while that of the borate buffer was 0.066. No statement of the effect of the ionic strength can be made on the basis of the present experimental results.

6.1.3 Effect of Packing Type and Size

The results comparing the two packing types have been presented in Table 2. Migration was observed in the 100-200 A0.5m packing. Migration was not measurable in the 100-200 P2 packing. However, during the migration experiments with 100-200 mesh P2 packing, two unusual observations were noted. A solid material formed at the column walls during elution. The

exact composition of the solid is unknown; however, it is suspected that the solid may be attributed to freezing of the buffer solution. This observation is consistent with the simultaneous observation that during the electrophoresis process, the outlet temperature of the cooling jacket was 0°C - below the 5°C level observed during other experiments. Data presented for the 100-200 mesh P2 packing are thus questionable. The results of this comparison, therefore, require repetition.

The results with two different sizes of packing have also been presented in Table 2. However, the results obtained with 100-200 mesh P2 packing are questionable as discussed previously, and no comparison is possible.

Experiments with the 200-400 mesh P2 packing were limited to 1000 v. For a measured current of 30 mA, a migration rate of 0.24 cm/min was observed; and for a 45 mA current, the observed migration rate was 0.32 cm/min. Since voltage and current are related by Ohm's Law, these results again indicate that magnitude of the voltage is important for mobility of a sample.

6.1.4 10-cm Column

Migration experiments were performed with the 10-cm column apparatus. In the column with glass frits, the applied voltage was limited to 360 v by electrical arcing throughout the column and in the electrode chambers above this voltage. No migration was observed below 360 v. These results indicate that the 10-cm column with glass frits is not suitable for the elution electrophoresis technique.

The 10-cm column was broken during the flow experiments and could not be used for migration experiments. The flow studies indicated potentially good resolution with the use of glass wool plugs to secure the packing. The 10-cm column with glass wool plugs is recommended for use in future investigations to examine the potential of operating at higher voltage gradients than those employed here.

6.2 Flow Experiments

6.2.1 10-cm Column

Flow studies were performed to determine the dispersion of an injected tracer as measured by the sample elution volume. The sample elution volume is calculated from the area under the curve produced by the UV recorder. The area was taken to be the product of the peak height times the width at one-half the peak height.

The results of flow studies performed with the 10-cm column with glass frits are presented in Fig. 10 for the 100-200 mesh P2 packing and the 200-400 mesh P2 packing. The data are scattered but suggest that better resolution is obtainable with the 200-400 mesh packing. Results were not

obtained with the -400 mesh P2 packing because the glass frits clogged with packing.

Injection of blue dye in the 10-cm column visually showed a stagnant region located in the area beyond the eluent tube. The output recorded simultaneously with the visual observation is shown in Fig. 13. The tail of the peak was due largely to holdup in the stagnant region. This suggests that a stagnant region also decreases the albumin sample resolution. The elimination of this stagnant region is recommended for future investigations.

One response curve was obtained with the 10-cm column using glass wool to secure the packing. This result showed a potentially good resolution. However, the column was subsequently broken and could not be used in further experiments.

6.2.2 40-cm Column

Flow studies were performed previously by Genung et al. (12) with the 40-cm column. Their results are presented in Figs. 11 and 12. The results of Fig. 11 for comparison of packing type suggest that the A-series packing gives better resolution than the P-series packing. The results of Fig. 12 for comparison of packing size predict that the 200-400 mesh packing gives better resolution than the 100-200 mesh packing. The combined result suggests that the best resolution will result with the 200-400 mesh A0.5m packing.

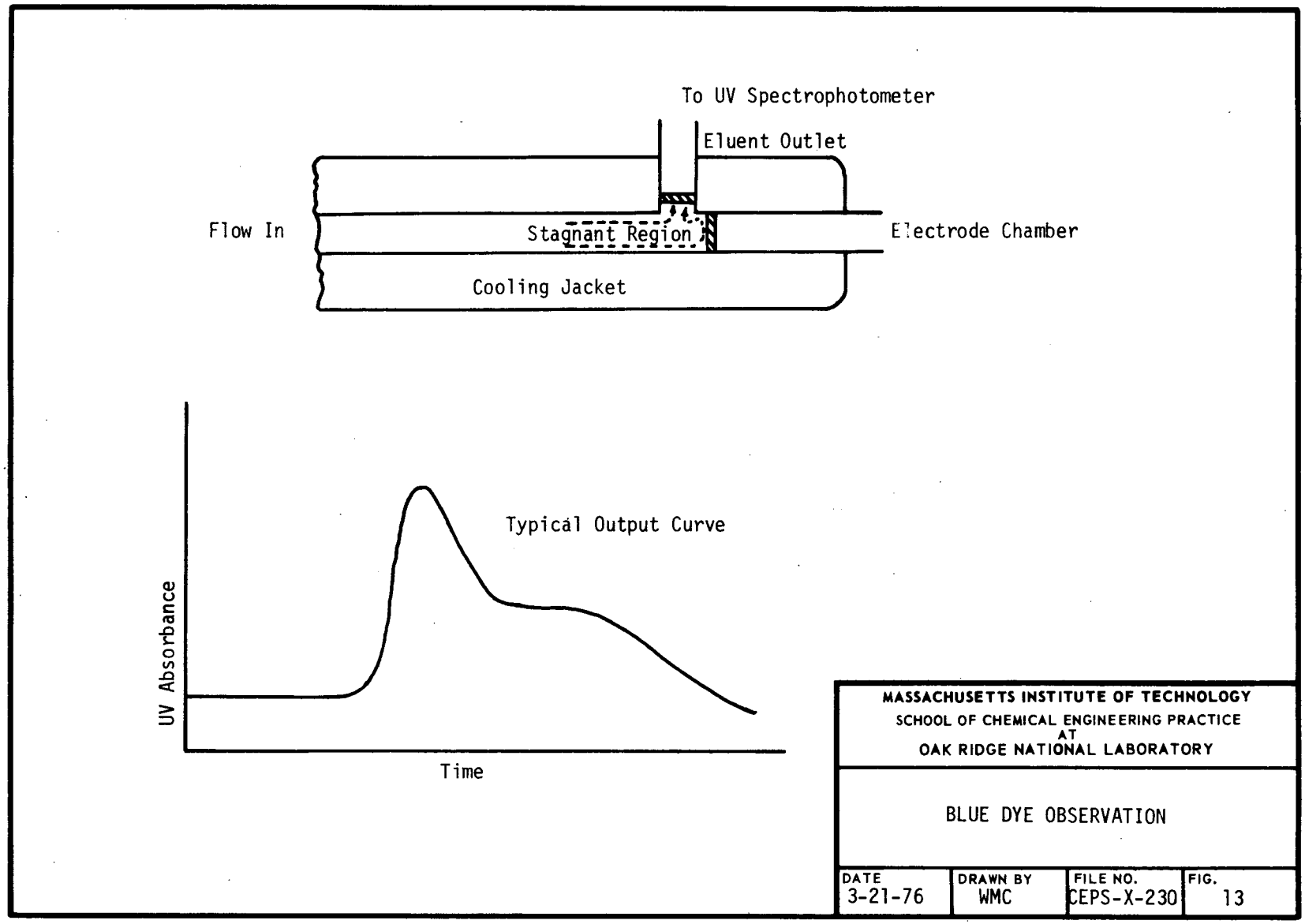
6.2.3 Comparison of Flow Experiments and Dispersion Theory

Several of the flow experiment response curves have been reduced to dimensionless coordinates, as suggested by Levenspiel (11) for comparison with dispersion theory. The calculational procedure is demonstrated in Appendix 10.1.3.

The curve presented in Fig. 14 shows a typical response curve from the 40-cm column. The curve appears sharply peaked for the rising portion, indicating little dispersion. The falling portion of the curve appears to tail slightly indicating an intermediate amount of dispersion as shown in the reference curve of Fig. 14. The indication of small dispersion for the rising portion suggests that the elution procedure does not introduce a large degree of sample dispersion. The tailing portion of the curve indicates that the stagnant region produces some dispersion of the sample.

6.3 Resistance Measurements

The results of the resistance measurements have been presented in Table 3. Each resistance is the mean value of several measurements. Comparison of these results in the 10-cm column containing tris borate buffer, both with and without glass wool, shows that the difference between the two results is about 1000 ohm. Similarly, results for the phosphate buffer, both with and without glass wool, show a resistance difference of 2 kΩ.


To UV Spectrophotometer
Eluent Outlet
Flow In
Stagnant Region
Electrode Chamber
Cooling Jacket
Typical Output Curve
UV Absorbance
Time
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AT
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BLUE DYE OBSERVATION
DATE 3-21-76
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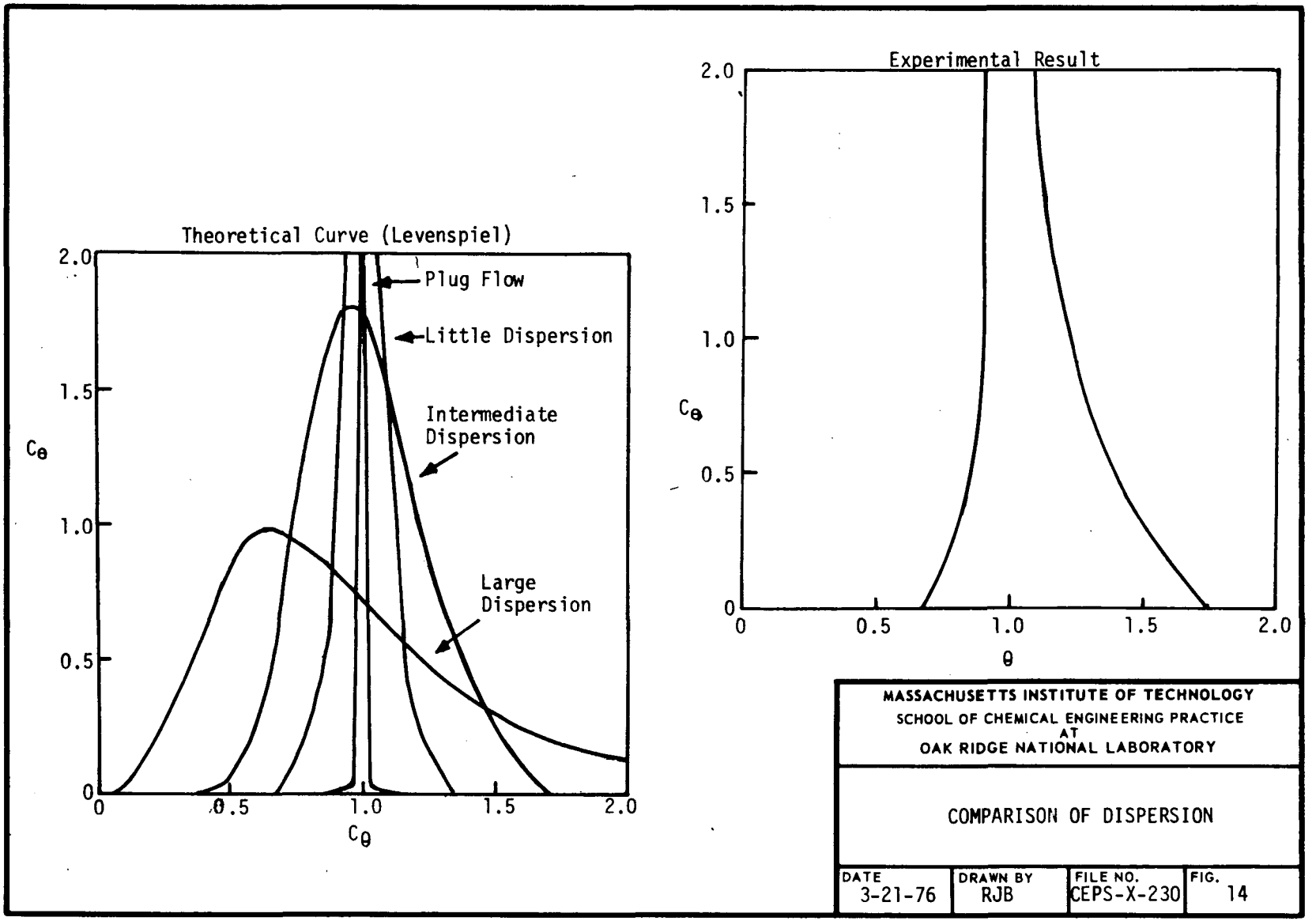
FIG. 13


Theoretical Curve (Levenspiel)
Plug Flow
Little Dispersion
Intermediate Dispersion
Large Dispersion
C_θ
C_θ
0
0.5
1.0
1.5
2.0
Experimental Result
C_θ
θ
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COMPARISON OF DISPERSION
DATE 3-21-76
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FILE NO. CEPS-X-230
FIG. 14

Since electrical resistances in series are additive, the glass wool resistance is approximately 1-2 kΩ. Comparison of the tris borate buffer, with and without glass frits, shows a difference of 31 kΩ that must be attributed to the frits. Comparison of the results for tris borate solution alone and phosphate solution alone indicates a difference in the resistance of the two solutions of 50 kΩ.

Although it is important to minimize the operating voltage for safety and operational simplicity, a high voltage gradient is required to produce migration. An increase in voltage gradient by reduction of the column length is thus desired.

Experimentally measured values of current for experiments in which migration occurred were 20-50 ma. Heat removal was an operational problem. Ohm's Law predicts that the heat produced by the passage of current is proportional to the resistance and the square of the current. The current through the frits and through the packing are the same. Hence, the use of glass wool instead of glass frits in the current path to reduce localized heating at the frits during electrophoresis is recommended.

6.4 Present Elution Electrophoresis Technique

The criteria for the demonstration of a suitable operating technique are the establishment of different electrophoretic mobilities for different sample components, thereby separating the components and the maintenance of this separation during the subsequent elution process. The analysis of the serum protein sample in which an applied voltage of 1000 volts was maintained for 30 min to the 40-cm column containing 200-400 mesh P2 packing and phosphate buffer, produced only one peak on the recorder output. At least five peaks corresponding to each of the major components, albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin, were expected. The presence of only one peak instead of the expected five peaks indicates that separation of the sample was not achieved. Thus, the present technique is not suitable. However, no conclusion discarding the concept of elution electrophoresis as a clinical tool is reached, since several experimental difficulties limit the operation of the present apparatus.

In addition, during experiments with samples of three hemoglobins, for which electrophoresis was maintained for a period of 30 min with an applied voltage of 1000 v in the 40-cm column containing 200-400 mesh P2 packing and phosphate buffer, migration was not observed for samples of pure hemoglobin, and separation was not apparent with the mixture of the three hemoglobins. This supports the conclusion that the present technique is not suitable for analysis of these mixtures.

6.5 Suggestions for Improving Column Performance

1. The glass frits should be replaced by glass wool since the glass wool has lower electrical resistance to current flow.

2. The cooling capability of the columns should be increased. It is suggested that the cooling water temperature be increased to 5°C from the present 0°C to avoid freezing, and the pumping capacity be increased to remove the heat to allow operation at higher voltages.

3. The column design should be modified as shown in Fig. 15 to incorporate the following improvements:

a. The sample is injected through a permeable septum located directly above the packed section of the column. This modification eliminates the backup that occurs in the present sample injection port.

b. The exit port for the eluent is in line with the column. This eliminates the stagnant region that develops in the present column due to the 90° turn at the exit port.

c. The eluent exit port is conical in shape, and the packing is extended into the contracted section of the cone. This contraction minimizes the mixing effects in the eluent outlet line leading to the UV spectrophotometer.

d. Separate inlet ports are provided for packing, inlet eluent flow, and packing washing. This improves the speed of experimental work and minimizes the possibility of introducing air bubbles into the apparatus.

7. CONCLUSIONS

1. The largest migration rate for albumin samples was observed with 200-400 mesh P2 packing, phosphate buffer, and an applied voltage of 1000 v.

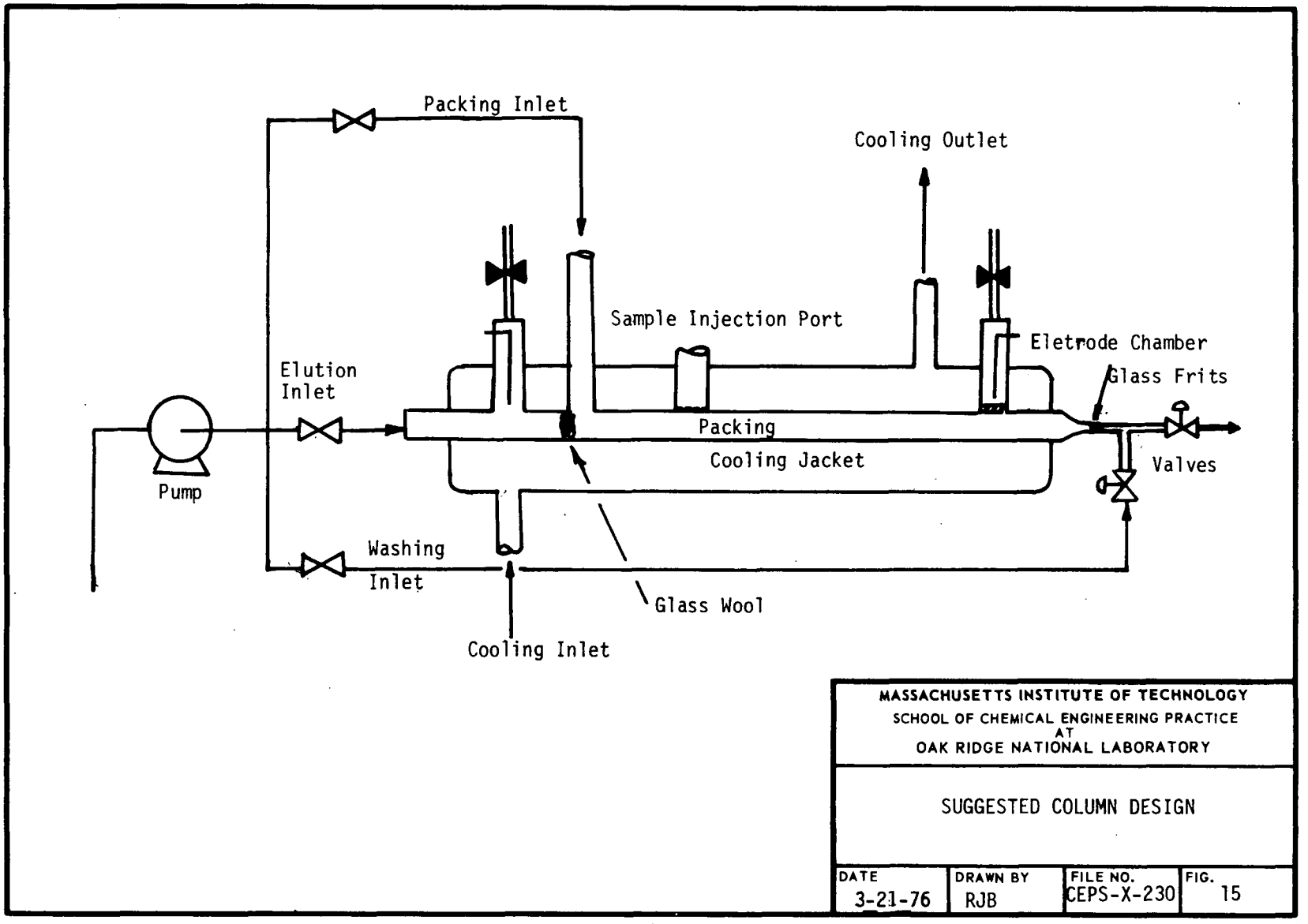
2. The operating conditions found to give the best migration rate for albumin did not produce separations of hemoglobin mixtures or serum proteins.

3. The configuration of the existing 10-cm column results in a high degree of dispersion attributable to the stagnant region near the column exit. The column design of the 40-cm column results in little dispersion of the sample during elution.

8. RECOMMENDATIONS

1. More experiments should be performed to determine the relationship of electrophoretic mobility to packing type and mesh size.

2. The suggestions for the new column design should be implemented and further experiments should be performed



MASSACHUSETTS INSTITUTE OF TECHNOLOGY SCHOOL OF CHEMICAL ENGINEERING PRACTICE AT OAK RIDGE NATIONAL LABORATORY			
SUGGESTED COLUMN DESIGN			
DATE 3-21-76	DRAWN BY RJB	FILE NO. CEPS-X-230	FIG. 15

9. ACKNOWLEDGMENT

The authors would like to thank R.K. Genung for his invaluable assistance and inspiration throughout the project and R.C. Lovelace for his technical advice in the experimental work.

10. APPENDIX

10.1 Sample Calculations

10.1.1 Experimental Migration Rate of Albumin

The volume of buffer solution required to wash the sample from the column without electrophoresis, V_1, is found from the equation as shown in Fig. 16.

$$V_1 = \frac{L_1 E}{S}$$

for experiment 45 conducted with 200-400 mesh P2 packing in phosphate buffer

$$V_1 = \frac{(8.65 \text{ cm})(0.167 \text{ ml/min})}{(1.27 \text{ cm/min})} = 1.14 \text{ ml}$$

Similarly,

$$V_2 = \frac{(11.25 \text{ cm})(0.0667 \text{ ml/min})}{(1.27 \text{ cm/min})} = 0.59 \text{ ml}$$

The migration rate is

$$v = \frac{4(V_1 - V_2)}{\alpha(\pi D^2)t_{el}} = \frac{4(1.135 - 0.591 \text{ ml})}{0.4(3.1416)(0.430 \text{ cm})^2(30 \text{ min})} = 0.32 \text{ cm/min}$$

10.1.2 Theoretical Migration Rate of Albumin

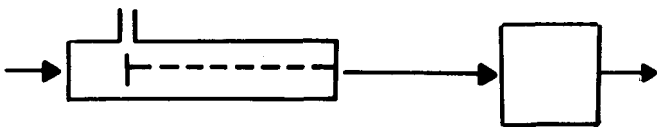
The Henry equation for free electrophoresis gives the migrating species mobility as

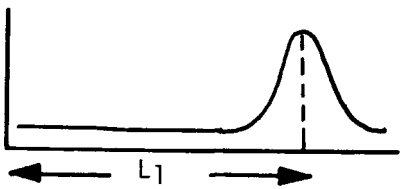
$$U = \frac{v}{E} = \frac{Z e}{6\pi\mu R} \frac{X_1(\kappa R)}{1 + \kappa R}$$

where κ is the Debye constant given by

$$\kappa = \left(\frac{8\pi N_o e^2}{1000\ \varepsilon kT}\right)^{1/2} I^{1/2}$$

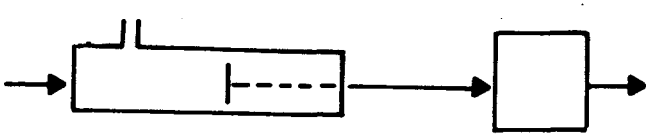
ELUTION ONLY

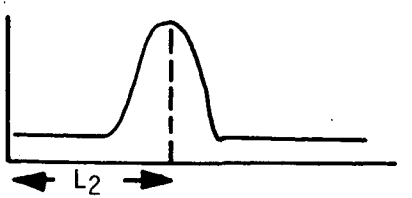




$$V_1 = \frac{L_1 E}{S}$$

ELUTION WITH ELECTROPHORESIS





$$V_2 = \frac{L_2 E}{S}$$

$$\text{Migration rate} = \frac{4(V_1 - V_2)}{\alpha(\pi D^2)t_{el}}$$

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MIGRATION RATE MEASUREMENTS

DATE	DRAWN BY	FILE NO.	FIG.
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and where $X_1(\kappa R)$ is the Henry function. For an albumin molecule with a 36 Å radius in the phosphate buffer solution, the term κR is very much less than one. Thus, the value of the Henry function is 1.0, the limiting value at low values of κR. Hence,

$$U = \frac{v}{E} = \frac{Ze}{6\pi\mu R}$$

If the albumin molecule is assumed to carry a net charge of 10, then

$$U = \frac{(10)(1.6 \times 10^{-19} \text{ coul})}{6\pi(2 \times 10^{-3} \text{ Pa-sec})(36 \times 10^{-10} \text{ m})} = 1.17 \times 10^{-8} \text{ m}^2/\text{V-sec}$$

and if the applied voltage gradient is 2500 V/m,

$$v = UE = (1.17 \times 10^{-8} \text{ m}^2/\text{V-sec})(10^3 \text{ V/m}) = 2.92 \times 10^{-5} \text{ m/sec}$$

$$= 0.18 \text{ cm/min}$$

10.1.3 Sample Elution Volume

The sample elution volume, V_e, is based on the response curve that appears on the strip chart recorder as shown in Fig. 17. The band width at one half the peak height, $L_{1/2}$, is measured and the elution volume is calculated.

$$V_e = \frac{EL_{1/2}}{S}$$

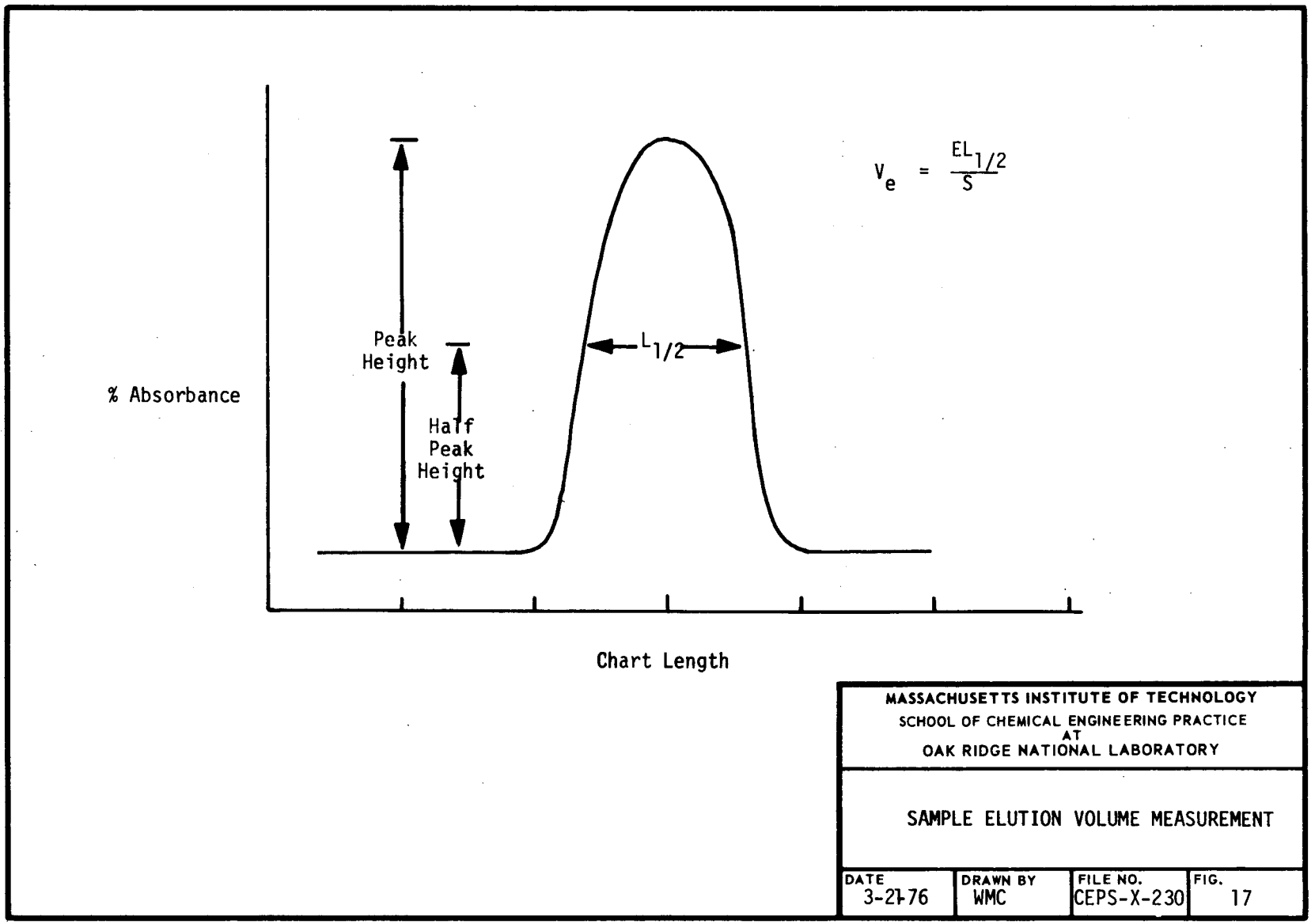
From the data of experiment 37,

$$V_e = \frac{(0.19 \text{ ml/min})(0.95 \text{ cm})}{(1.27 \text{ cm/min})} = 0.14 \text{ ml}$$

10.1.4 Dispersion Model Variables

The residence time of the fluid flow in the packed column can be calculated by:

$$\bar{t} = \frac{\alpha(\pi D^2)L}{4E}$$


$V_e = \frac{EL_{1/2}}{S}$
% Absorbance
Peak Height
Half Peak Height
$L_{1/2}$
Chart Length
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OAK RIDGE NATIONAL LABORATORY
SAMPLE ELUTION VOLUME MEASUREMENT
DATE 3-21-76
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FIG. 17

For the data of experiment 45,

$$\bar{t} = \frac{(0.4)\pi(0.43 \text{ cm})^2(20 \text{ cm})}{(4)(10.8 \text{ ml/hr})(1 \text{ hr}/60 \text{ min})} = 6.44 \text{ min}$$

The time variable in flow experiments can be reduced to a dimensionless time variable with the equation

$$\theta = \frac{t}{\bar{t}}$$

For the data of experiment 45 at the fourth data point,

$$\theta = \frac{5.84 \text{ min}}{6.44 \text{ min}} = 0.907$$

The area under the elution curve can be evaluated using the trapezoidal rule. The area under a section of the curve between times t_i and t_{i+1} where the concentrations are C_i and C_{i+1} respectively, is given by

$$\Delta Q_i = (t_{i+1} - t_i)\left(\frac{C_{i+1} + C_i}{2}\right)$$

and

$$Q = \sum_{i=1}^{n} \Delta Q_i$$

From the data of experiment 45

$$\Delta Q_3 = (5.84 - 5.8 \text{ min})\left(\frac{0.32 + 0.22}{2} \text{ in.}\right) = 0.0108 \text{ in.-min}$$

The concentration at any point can be reduced to a dimensionless concentration variable, $\bar{C}$, by

$$\bar{C} = \bar{t}\left(\frac{C_t}{Q}\right)$$

From the data of experiment 45, time is 5.84 min,

$$\overline{C} = 6.44 \text{ min}\left(\frac{0.32 \text{ in.}}{4.424 \text{ min-in.}}\right) = 0.466$$

10.1.5 Phosphate Buffer Ionic Strength

For the 0.25 M Na_2HPO_4 buffer solution used in the experiments, the ionic strength is calculated as

$$I = \frac{1}{2} \sum_i C_i Z_i^2$$

where C_i is an ionic species concentration and Z_i is the species charge. Normally in solution, one would expect to find Na^+, H^+, OH^-, PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$. From the dissociation constants of phosphoric acid, one finds

$$\frac{(H_2PO_4^-)(H^+)}{(H_3PO_4)} = 8.9 \times 10^{-3}$$

$$\frac{(HPO_4^{2-})(H^+)}{(H_2PO_4^-)} = 4.8 \times 10^{-5}$$

$$\frac{(PO_4^{3-})(H^+)}{(HPO_4^-)} = 2 \times 10^{-13}$$

Since the buffer solution pH was 8.9, the hydrogen ion concentration was

$$(H^+) = 1.26 \times 10^{-9}$$

Thus,

$$\frac{(H_2PO_4^-)}{(H_3PO_4)} = 7 \times 10^6$$

$$\frac{(HPO_4^{2-})}{(H_2PO_4^-)} = 3.8 \times 10^4$$

and

$$\frac{(PO_4^{3-})}{(HPO_4^{2-})} = 1.6 \times 10^{-4}$$

Thus the concentration of HPO_4^{2-} will be at least four orders of magnitude larger than the concentrations of phosphoric acid, $H_2PO_4^-$ ion or PO_4^{3-} ion. Thus one would expect complete dissociation of the Na_2HPO_4 salt into Na^+ ions and HPO_4^{2-} ions only, and the ionic strength may be calculated as

$$I = \frac{1}{2} \sum_i C_i Z_i^2$$

$$= \frac{1}{2}[(0.50 \text{ mole } Na^+/\ell)(1)^2 + (0.25 \text{ mole } HPO_4^{2-}/\ell)(2)^2]$$

$$= 0.75 \text{ moles}/\ell$$

10.2 Location of Original Data

All recorded output, operating conditions, and pertinent observations for each experiment are collected and recorded in ORNL Databook A-7650-G, pp. 1-47, in the possession of R.K. Genung at Oak Ridge National Laboratory. All calculations of values pertaining to discussion of results are collected in a calculation file located at the MIT School of Chemical Engineering Practice School, Bldg. 3001, ORNL.

10.3 Nomenclature

C_t	recorder representation of sample concentration in the UV spectrophotometer at any time t, in.
$\bar{C}$	reduced concentration, dimensionless
D	column diameter, cm
e	fundamental unit of charge (1.60910×10^{-19} coulomb)
E	elution flow rate, ml/min
E	electric field strength, V/cm
I	buffer solution ionic strength, mole/liter
k	Boltzmann's constant
L	length of column from injection port to outlet glass frit (cm)

L_1	distance on strip chart from start of elution to peak without electrophoresis, cm
$L_{1/2}$	band width at half peak height, cm
L_2	distance on strip chart from start of elution to peak after electrophoresis, cm
N_o	Avogadro's number
Q	integrated area under concentration-time curve, in.-min
r	column radius, cm
R	sample particle radius, cm
S	recorder chart speed, cm/min
$\overline{t}$	buffer solution column residence time, min
t	time, min
t_{el}	electrophoresis time, min
U	electrophoretic mobility, $cm^2/V\text{-}sec$
v	sample migration rate, cm/sec
V_1	volume of buffer solution required to wash a sample from the column with no electrophoresis, ml
V_e	elution volume, ml
$X_1(\kappa R)$	Henry's function, dimensionless
Z	charge number, dimensionless
α	packing void fraction, dimensionless
ϵ	dielectric constant
κ	reciprocal Debye length, cm^{-1}
θ	reduced time, dimensionless
μ	viscosity of buffer solution, poise

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