

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

USE OF SCINTILLATION FLOW CELL IN PREPARATIVE
PURIFICATION OF C-14 LABELED PHOSPHONATES

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Monsanto Company relies heavily on use of radiolabeled materials in obtaining regulatory environmental assurance on its products. The ability to do rapid, economical purity assessments and preparative scale purification of tagged products has become highly important. Liquid chromatography is the preferred preparative technique when applicable because it is versatile and relatively safe for processing multimillicurie amounts of radioactivity. A noteworthy deficiency, however, has been availability of a suitable, inexpensive commercial radioactivity flow detector. The lack of such a detector often necessitates use of time-consuming fraction collection and liquid scintillation counting of many samples for analysis and purification.

This paper describes use of anion exchange column chromatography to prepare subgram quantities of pure carbon-14 labeled phosphonates. Use of a new, low cost commercial scintillation flow cell permitted detection of radiolabeled components under conditions where refractive index and ultraviolet detectors were not usable.

I. INTRODUCTION

Monsanto Company relies very heavily on the use of high purity radiolabeled materials in obtaining regulatory environmental assurance data on its products. The ability to do rapid economical radiochemical purity assessments and preparative scale purification of tagged products that are often

complex in nature has become highly important. Liquid chromatography systems (simple column, GPC or HPLC) because of their versatility, advanced development, convenience and reliability, and radiochemical safety advantages make these the preferred preparative separation tools. A noteworthy deficiency in liquid chromatography of radiolabeled materials has been a simple, sensitive, inexpensive and reliable commercial radioactivity flow detector. The lack of such a detector often necessitates use of the costly, labor intensive alternative of fraction collection, aliquotting, liquid scintillation counting and post-analysis fraction compositing, etc. to develop separation methods and isolate purified fractions.

This paper describes the use of a prototype inexpensive commercial on-stream radioactivity detector (1) utilizing a scintillator-glass-packed flow cell for radiochemical purity analysis and preparative purification of carbon-14 labeled phosphonate materials under chromatographic conditions where conventional LC detectors were not successful.

II. MONSANTO PHOSPHONATE CHEMICALS

The chemical structures of the radiolabeled phosphonate materials purified in this study are given in Figure 1. These materials have become industrially significant in various water treatment applications including:

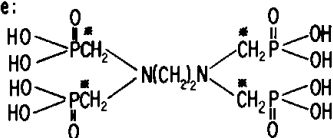
- scale inhibition
- crystal growth modification
- sludge modification
- chelation
- sequestration
- metal ion control
- dispersion
- deflocculation
- corrosion inhibition
- surface modification

The products are marketed under Monsanto's registered trademark DEQUEST[®]. Additional DEQUEST materials in addition to those shown in Figure 1 are available. Properties, applications and chemical structures vary significantly among the different products and detailed information is available from a series of technical pamphlets (2). These products will be hereafter referred to by their Monsanto tradenames which are simpler and more convenient to use than the chemical names or acronyms.

DEQUEST[®] 2041 Phasphanate

Chemical Name: Ethylenediaminetetra (methylenephosphonic Acid)

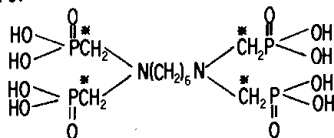
Structure:



DEQUEST[®] 2051 Phasphanate

Chemical Name: Hexamethylenediaminetetra(methylenephosphonic Acid)

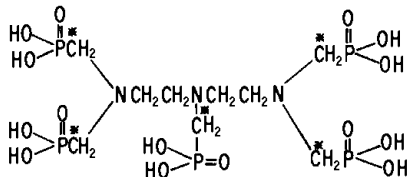
Structure:



DEQUEST[®] 2060 Phasphanate

Chemical Name: Diethylenetriaminepenta (methylenephosphonic acid)

Structure:



* Position of Carbon -14 label

Fig. 1. Monsanto Carbon-14 Labeled Phosphonates Purified at Preparative Scale.

III. RADIOCHEMICAL PURITY PROBLEMS WITH SYNTHESIZED CARBON-14 LABELED PHOSPHONATES

Gram scale radiolabeled syntheses of these three products were performed using a Mannich-like reaction in which phosphorous acid combines with Carbon-14 labeled formaldehyde and the particular amines required. The environmental application required several millicuries of carbon-14 radioactivity for each phosphonate at specific activities of several millicuries per millimole and high radiochemical purities. We were not successful in producing any of the final products shown in high yields but obtained mixtures containing significant amounts of what appeared to be all the possible intermediate incompletely phosphonated compounds. The separation properties of intermediates and final products were found to

be quite similar as determined by TLC on cellulose plates and anion exchange column chromatography.

IV. FRACTIONATION OF IMPURE RADIOLABELED PHOSPHONATE REACTION MIXTURES BY ANION EXCHANGE CHROMATOGRAPHY

A. Use of Simple Column Chromatography, Fraction Collection and Liquid Scintillation Counting

Our first efforts at preparative purification were made on an impure DEQUEST 2060 ^{14}C mixture. We began quite simply since at this time we did not have a radioactivity flow detector capability. A small, gravity flow, open anion exchange bed 12.6 mm dia. ID and 12 cm long was used containing 10 grams of Bio-Rad AG 1-X 8, 200-400 mesh anion exchange resin converted from chloride to carbonate form. Elution of a 10 mg sample of impure DEQUEST 2060 ^{14}C at low specific activity was explored using a series of aqueous ammonium carbonate solutions made up in increasing concentration increments of 0.1 molarity and sequentially applied to the column. Effluent was collected directly into liquid scintillation count vials by an ISCO Model 328 fraction collector. The chromatogram appears in Figure 2. The results encouraged us:

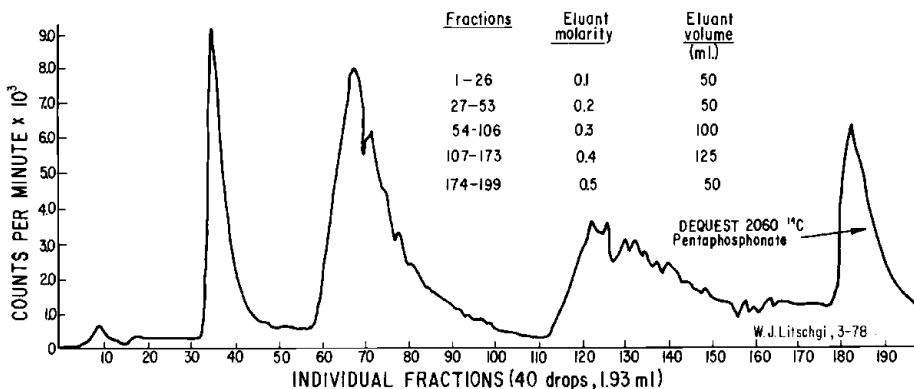


Fig. 2. Impure DEQUEST 2060- ^{14}C Pilot Scale Fractionation, Simple Anion Exchange Column Technique

- Applicability of ammonium carbonate for component separation was demonstrated and the approximate concentration range needed for elution of the various components was learned.
- Ammonium carbonate efficiently decomposed into volatile products at 70-80°C under water aspirator vacuum of 30-100 mm leaving products uncontaminated with eluant.
- A significant improvement in radiochemical purity of the DEQUEST 2060 ^{14}C pentaphosphonate from 20% to more than 80% resulted.
- A recovery of nearly 95% of total carbon-14 radioactivity applied to the column was obtained.
- Preparative scale-up appeared to involve primarily an appropriate increase in column diameter and length.

Several scale-up experiments were attempted with 500 mg samples using larger resin charges in columns having greater diameter and bed length. Our best (and final) scale-up attempt with the open gravity flow system used a resin charge of 75 grams in a bed 22 mm diameter and 26 cm long. An examination of the radioactivity separation chromatogram given in Figure 3 illustrates the several fundamental problems and limitations of this simple approach.

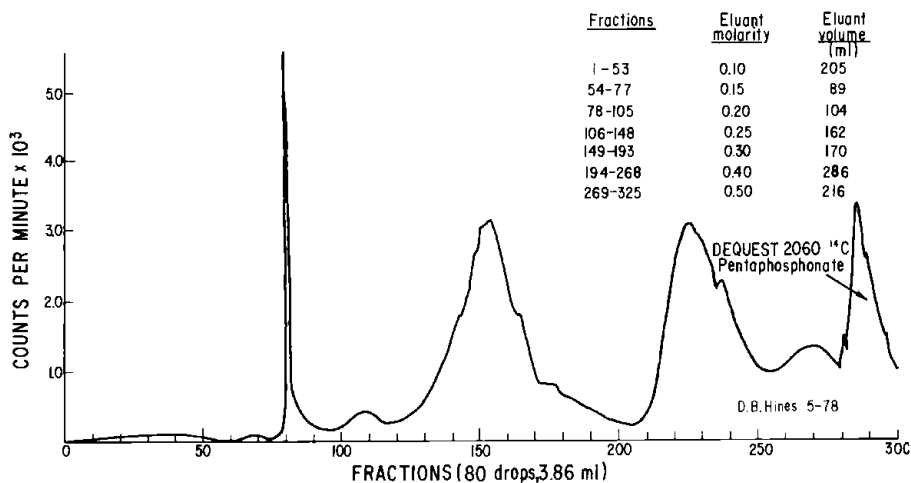


Fig. 3. Impure DEQUEST 2060- ^{14}C Preparative Scale Fractionation, Simple Anion Exchange Column Technique

- Separation between DEQUEST 2060 ^{14}C tetra- and pentaphosphonates was still inadequate. Maximum radiochemical purity of about 95% was obtainable but at considerable sacrifice in yield.
- The method was inefficient, tedious and costly. The large number of samples (325) per run and the necessity to stop elution and measure radioactivity in collected fractions by liquid scintillation counting to evaluate success of different elution strategies required several days work to perform a single experiment.
- Finally, achievement of 99% radiochemical purity required two tedious runs--the first to fractionate the major impurities and the second to remove the residual tetraphosphonate from the final product.

At this point, we abandoned the open column system.

B. Use of Automated Column Chromatography, Isocratic Elution and Differential Refractometry

Our second approach utilized a versatile commercial liquid chromatography column (3) readily adaptable for use with HPLC components and packed with 100 grams of the same anion exchange resin in carbonate form used previously forming a bed 25 mm in diameter and 33 cm long. The column was operated using Waters Associates HPLC pumps, sample injection components and differential refractometer detector. This set-up provided greater convenience, experimental reproducibility and much higher eluant flow rates permitting faster test evaluations. However, isocratic elution with ammonium carbonate did not adequately separate the DEQUEST 2060 ^{14}C tetra- and pentaphosphonate components. Gradient elution appeared necessary to improve component separation but it complicated detection of eluted components. Our differential refractometer was not usable under gradient conditions and these phosphonate materials had no useful UV absorbance or fluorescence properties. So we were again confronted with fraction collecting and liquid scintillation counting.

C. Use of Automated Column Chromatography, Gradient Elution and Radioactivity Flow Cell

Fortunately, at about this time, we had obtained on loan the prototype radioactivity flow detector. This type detector may be used satisfactorily under gradient elution conditions. After solving some initial problems with flow cell

design and leakage, we achieved success in pilot scale separations of the impure DEQUEST 2060 radiolabeled components using the automated column chromatography previously described. A pilot scale separation of the impure DEQUEST 2060 ^{14}C mixture appears in Figure 4 and a preparative scale separation in Figure 5. Results were a significant improvement over our previous efforts:

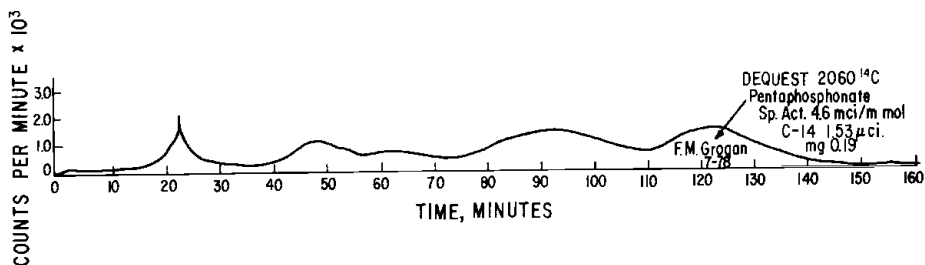


Fig. 4 Impure DEQUEST[®] 2060 ^{14}C Pilot Scale Fractionation Linear Gradient Elution Anion Exchange Technique

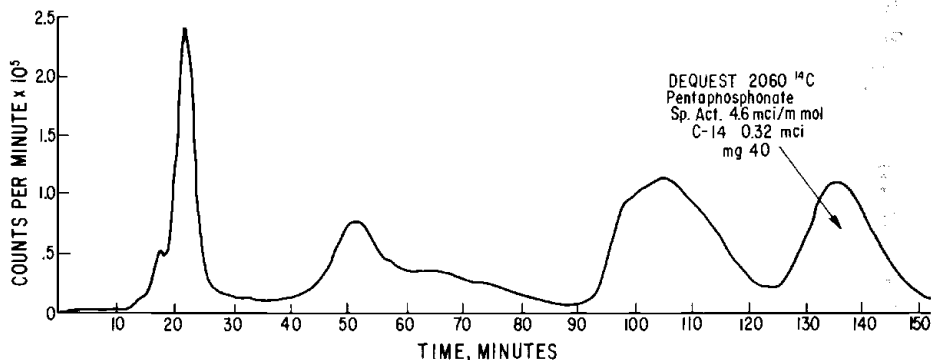


Fig. 5. Impure DEQUEST[®] 2060 ^{14}C Preparative Scale Fractionation, Gradient Elution Anion Exchange Column Technique

- The radioactivity flow detector with linear rate meter recording produced directly the radioactivity separation chromatogram. This eliminated much effort previously spent in fraction collection and radioactivity analysis of several hundred fractions to obtain this same information.

- The DEQUEST 2060 ^{14}C pentaphosphonate component separated to 95% radiochemical purity in a single pass with a minimum of yield sacrifice. Based on later work with the other phosphonates, we now feel that 99% radiochemical purity is probably obtainable on a single pass under a modified elution strategy. But we elected to composite several 95% pure fractions and put them through the columns a second time to reach the higher purity.

- Another significant improvement was the great shortening of time required per sample fractionation. Less than three hours was required to separate the sample mixture and two preparative scale runs per day were achieved. The initial approach took about 20 hours just to fractionate the same size sample and gave both lower purity and yield.

- Finally, the major radiolabeled phosphonate intermediates were also separated fairly cleanly and were collected and stored for future needs.

Preparative purification of radiolabeled DEQUEST 2041 and 2051 was done with the same equipment and by quite similar procedures. Typical radioactivity chromatograms for the two products are given in Figures 6 and 7 respectively.

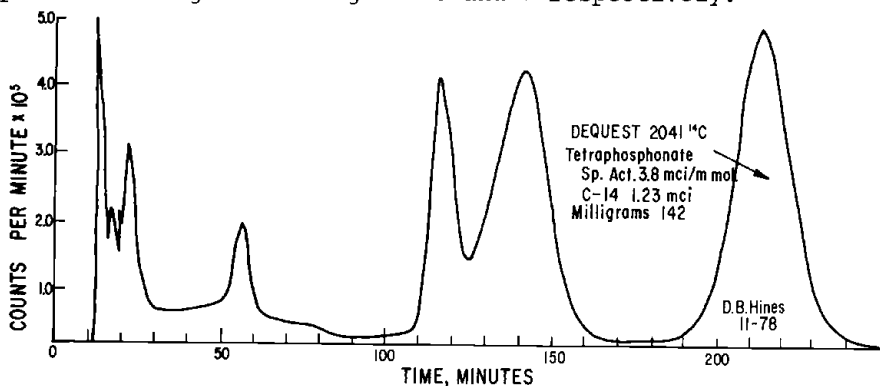


Fig. 6. Impure DEQUEST[®] 2041 ^{14}C Preparative Scale Fractionation, Gradient Elution Anion Exchange Column Technique

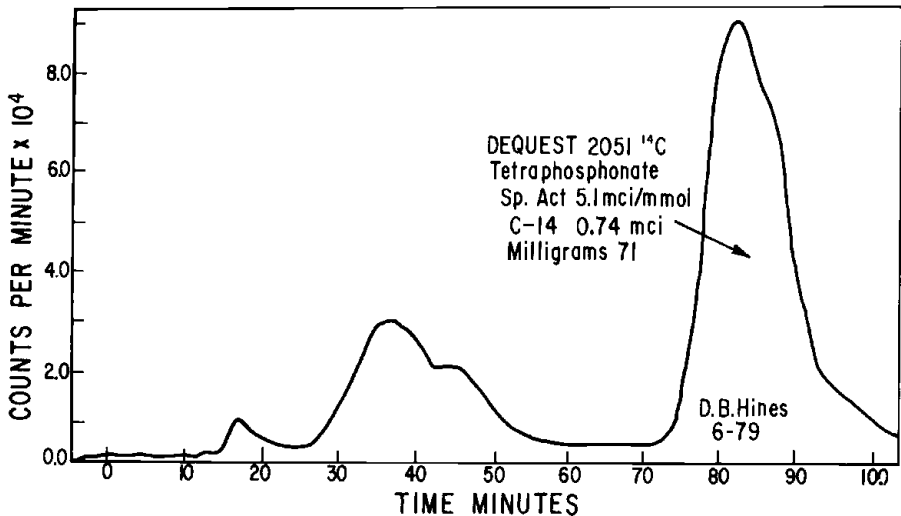


Fig. 7. Impure DEQUEST[®] 2051 ¹⁴C Preparative Scale Fractionation, Linear Gradient Elution Anion Exchange Technique

A typical preparative scale phosphonate fractionation consists of the following operations:

- Phosphonate acid sample neutralization (with solid ammonium carbonate, Fisher Catalog A-652, to cessation of carbon dioxide evolution, pH 8-9).
- Neutralized sample filtration (small pyrex Buchner funnel, fritted glass, fine porosity, Fisher Catalog 20-704C).
- Filtered sample injection (a 1.0 ml Precision Sampling Corporation PRESSURE-LOK Series "S" syringe, catalog no. 030033 fitted with a 0.0020 inch O.D. custom modification of catalog item no. 943051-B needle is compatible with Waters Associates HPLC sample injector U6K, has virtually no sample hold up or radioactivity leakage during handling).
- Linear gradient elution at 5 ml/min. with aqueous ammonium carbonate (blended from filtered, degassed deionized or distilled water and 1.0 molar ammonium carbonate. A column cleanup after each preparative separation with full strength 1.0 molar eluant is important).
- Collection of eluted fractions (8 ounce glass screw cap bottles are satisfactory. Refrigerated storage is recommended to reduce degradation of unstable ammonium salts if evaporation and acidification steps are delayed).

- Prompt evaporation down to a few milliliters of concentrated solution (same day as separated if possible, on a rotary film evaporator using a 70°C water bath and water aspirator vacuum of 100-150 mm mercury).
- Prompt acidification with strong acid cation exchange resin of the concentrated ammonium salt solution (direct acidification with *freshly washed* Bio-Rad AG 50-W-X8, 200-400 mesh, hydrogen form, to a pH of 1-2 and passage through a small column of the same resin is satisfactory).
- Prompt refrigerated storage (1% aqueous solutions of the acids are stable for several weeks. Freezer storage as evaporated solids is recommended for longer periods).

The experimental setup appears in Figure 8.

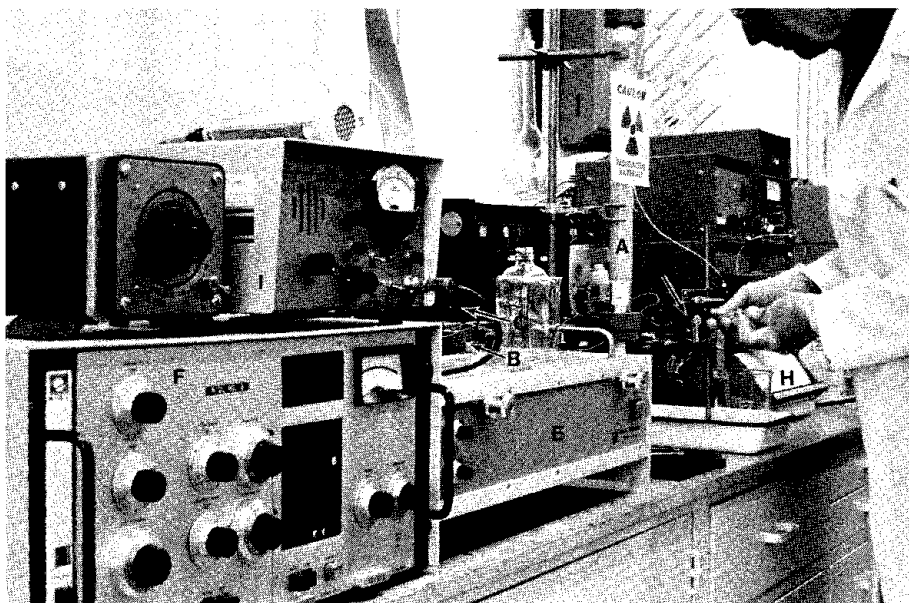


Fig. 8. Preparative Anion Exchange Column Chromatography Equipment

Label	Component
A	Pharmacia liquid chromatography column SR 25/45
B	Waters Associates HPLC Pumps, Model 6000A
C	Waters Associates HPLC Solvent Programmer, Model 660. Not shown are reservoirs of degassed and filtered water and 1 molar ammonium carbonate solution.
D	Waters Associates HPLC sample injector Assembly U6K with 2.0 ml sample loop.
E	Nuclear Enterprises LSM-1 radioactivity flow detector.
F	RIDL supplementary electronics (H. V. Supply, Scaler, Linear Ratemeter). Not shown is a Hewlett Packard 465A amplifier that supplanted the RIDL pulse height analyzer module.
G	Waters Associates Differential Refractometer R-401.
H	Hewlett Packard dual per recorder, Model 7132A.
I	Eberline GM Area Radiation Monitor, Model RM-19.

The very efficient Buchi/Brinkmann Rotovapor R film evaporator used to concentrate fractions is shown in Figure 9. We found this evaporator to be fast, versatile and trouble free.

V. THE RADIOACTIVITY FLOW DETECTOR

The Nuclear Enterprises, Inc. LSM-1 Radioactivity flow detector used for this work is a prototype design obtained on a loan evaluation basis (and since purchased). The instrument is conventional in design consisting of:

- a light-tight rectangular box with dimensions of 6 x 20.5 x 8.5 inches, weighing 45 pounds and having top and bottom external flow cell connections.
- dual photomultiplier tubes mounted face to face with spacing for flow cell insertion.
- electronic circuitry that performs signal summation, some amplification, coincidence screening and pulse height selection.

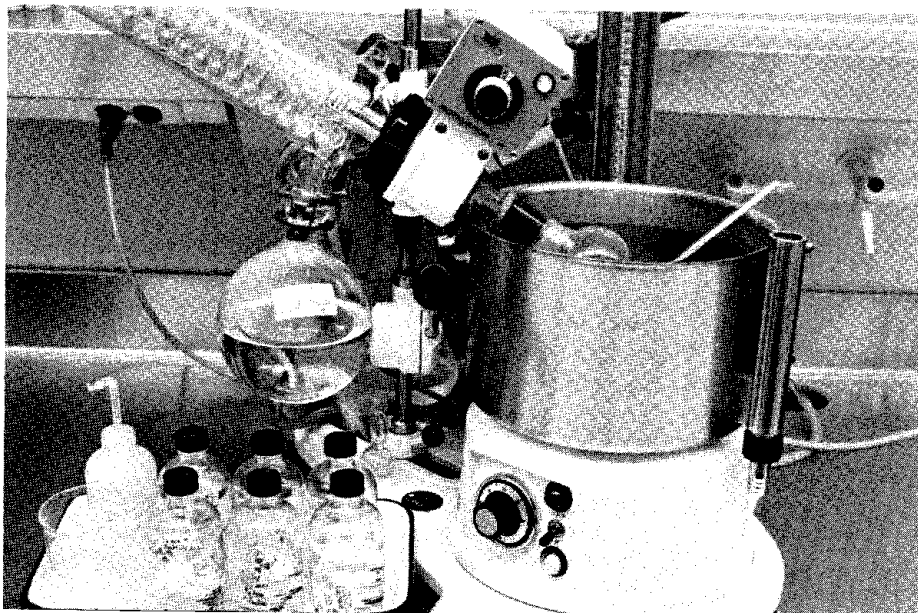


Fig. 9. Buchi/Brinkmann Rotovapor R Liquid Evaporator

- A flow cell with short straight glass tube packed with 0.25 - 0.50 mm scintillation glass (1) and having a liquid volume of about 150 microliters is optically coupled to the facing PMTs.
- A cylindrical lead shield 0.8 inches thick surrounds the flow cell with openings to admit the PMTs.

A block diagram of the electronic circuitry components of the LSM-1 is reprinted in Figure 10 by the permission of Nuclear Enterprises, Inc. The photomultiplier tubes are matched EMI 9805A, 2 inches in diameter, contain low potassium glass, are of venetian blind design, with a 40 nanosecond coincidence gate.

The LSM-1 requires several supplementary components as previously noted:

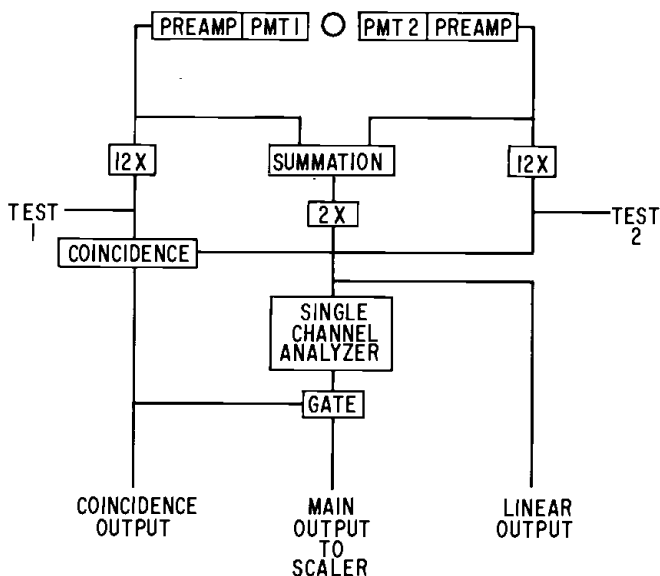


Fig. 10. Nuclear Enterprises Radioactive Flow Detector LSM-1 Block Diagram

- A high voltage supply for the PMTs. It must be capable of 0.2 MA at 2000 volts. This was provided by an RIDL Designer Series gamma single channel analyzer high voltage module, model 40-9B.
- An output signal display. This, likewise, was provided by modules in the RIDL unit (scaler, model 49-30 and linear rate meter, model 35-9). The rate meter output is registered either on a Hewlett Packard 7132A dual pen or an L and N Speedomax H single pen recorder.
- Supplementary amplification. This may be unnecessary with newer scaler/rate meter instrumentation. However, the LSM-1 output signal was insufficient to operate the RIDL linear rate meter and was, therefore, further amplified through a Hewlett Packard 465A instrument.

Several instrument specifications reprinted by permission of Nuclear Enterprises, Inc. are summarized in Table 1. Important items missing in the specifications are the counting efficiencies for Tritium and Carbon-14 and cell background. We determined the efficiency for Carbon-14 at about 20% in our rebuilt cell. We have done no work with Tritium. We found that the detector's radioactivity response does not reach a maximum with increasing high voltage but steadily increases. At a count efficiency of 20%, the background is ~100 cpm. Simplified details of the LSM-1 flow cell design are given in Figure 11 by permission of Nuclear Enterprises, Inc. The cell consists of:

Table 1. Nuclear Enterprises, Inc. Radioactivity Flow Detector LSM-1, Specifications

PHYSICAL

height	6 inches
width	20.5 inches
depth	8.5 inches
weight	45 pounds

ELECTRICAL

power supply	100-120 V AC, 60 Hz
power consumption	10 Watts Max
fuses	0.1 Amp
H.V.	up to 0.2 mA at 2000 V

OUTPUT SIGNALS

1. scaler	
amplitude	3.5 V logic pulse
polarity	Pos. or Neg. normally positive
width	1 microsecond
fan out	10
2. coincidence	
amplitude	3.5 V logic pulse
polarity	positive
width	500 nsec
fan out	10
3. linear	
gain	2
polariy	positive
decay time	1.2 microsecond
impedance	30 ohms

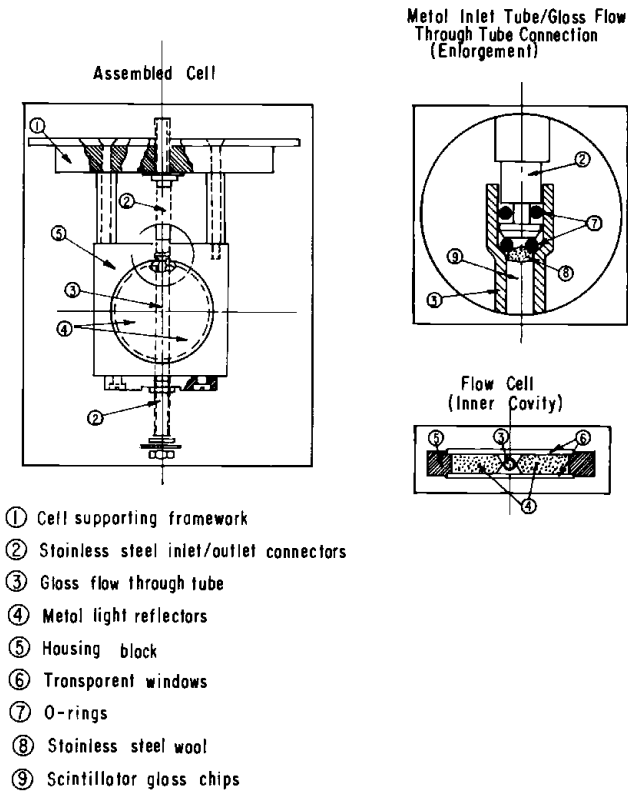


Fig. 11. Nuclear Enterprises, Inc. LSM-1 Prototype Flow Cell

- A pyrex glass inner flow-through tube charged with scintillator glass.
- A supporting framework that positions the flow cell rigidly between the sliding PMTs and makes a light-tight seal with the instrument case.
- Stainless steel inlet and outlet connector fittings.
- Two metal light reflectors with beveled edges positioned on each side of the flow-through tube.
- Housing block accommodating the flow-through tube and the two light reflectors.
- Transparent windows that seat into the outside faces of the housing block and make optical coupling with the PMTs.

The inner cavity surrounding the flow-through tube is filled with a clear optical jelly (Dow Corning Dielectric Gel Q3-6527 A and B).

The prototype flow cell provided with the LSM-1 that we obtained proved troublesome with pronounced susceptibility to fracture of the inner flow-through tube at the inlet/outlet connection. During the course of two rebuildings of the cell, several contributing causes for inner tube breakage were discovered:

- The enlarged ends of the flow-through tube lacked adequate shoulder to reliably seat the sealing O-ring and allowed it to slip sideways into the narrower packed section. Further tightening produced direct glass-metal contact.
- The threaded inlet/outlet connectors have no limiting stops. We found the small diameter tube awkward to tighten with fingers alone and with a small vise grip tool it is easy to over-tighten and stress the thin glass.
- The top plate tapping and threading were slightly off perpendicular and the top connector has a slight wobble motion. This wobble is sufficient to stress the flow-through tube during dismantling and assembly operations since the glass tube is held fairly rigidly by the optical inner sealant.

Several other features of the cell complicated rebuilding:

- The optical sealant filling the inner cavity proved to also be a tenacious glue making removal of the covering windows difficult.
- Organic solvents able to soften the inner sealant also fogged the plastic transparent windows and softened the housing block which was also plastic.

We made several minor modifications in rebuilding the flow cell that have proven satisfactory:

- The inner flow-through tube was replaced with a design fabricated at our research glass shop. The enlarged end sections were made heavier and a better inner O-ring shoulder was produced by careful internal grinding with a carbon mandrel.
- Quartz windows also produced by the glass shop replaced the plastic ones.
- Kal-Rez O-rings replaced the originals which swelled shut upon contact with acetone solvent.

We have also demonstrated that a fast flush of the cell flow-through tube with 10% aqueous hydrofluoric acid followed by prompt water rinsing cleanly removes radioactive materials adsorbed on the scintillator glass restoring background to normal levels. In preparative work with millicurie levels of carbon-14 labeled phosphonates, cell background occasionally became as high as 3000-4000 cpm.

VI. CONCLUSION

We believe this application is noteworthy for several reasons:

- Anion exchange column chromatography with ammonium carbonate eluant appears to have considerable potential for effective separations of mixtures of phosphonate materials. This utility would be increased by availability of LC detectors able to better function in gradient elution applications.
- An inexpensive commercially available radioactivity flow detector (with improved flow cell) has been demonstrated to be practical and reliable over several months of continuous application and is still in operation.

- Continuous radioactivity flow monitoring has very significantly reduced the cost of development of separation methods and production of useful amounts of high purity phosphonate radiochemicals. It has also permitted simple application of powerful LC gradient elution techniques to fractionation of hard-to-separate radioactive mixtures.

- A practical method is outlined for performing separations of three specific radiolabeled phosphonates at radioactivity levels ranging from a few microcuries to more than three millicuries at sample loads up to about 500 milligrams.

ACKNOWLEDGMENTS

Acknowledgment is gratefully made to R. J. Daniels of Monsanto Agricultural Products Company Research Department for suggestions on chromatographic separations of phosphonate materials.

SOURCES

1. Nuclear Enterprises, Inc., 930 Terminal Way, San Carlos, California 94070, Phone (415) 592-8663.
2. Monsanto Company Technical Bulletins:
IC/SCS-320 DEQUEST[®] 2000 and 2006 Phosphonates
IC/SCS-321 DEQUEST[®] 2041 and 2051 Phosphonates
IC/SCS-322 DEQUEST[®] 2060 Organophosphorus Product
IC/SCS-323 DEQUEST[®] 2010 Phosphonate

Available from

Monsanto Industrial Chemicals Company
800 N. Lindbergh Blvd.

St. Louis, Missouri 63166

ATTN: J. M. Kuenz, B3SG

Phone: (314) 694-1000

3. Pharmacia SR 25/45 available from
Pharmacia Fine Chemicals AB
Box 175
S-75104 Uppsala 1, Sweden
Phone 018/15 56 60

or

Pharmacia Fine Chemicals
Division of Pharmacia, Inc.
800 Centennial Avenue
Piscataway, N. J. 08854
Phone (201) 469-1222

DISCUSSION

*Details of Gradient Application to the DEQUEST[®] Products
Preparative Purification*

The ammonium carbonate/water eluant flow rate was 5 ml/min and the gradient mode was linear for each application. The gradient composition range and duration, however, differed:

<i>Application</i>	<i>Eluant Gradient</i>	
	<i>Composition Range (Molarity)</i>	<i>Duration (Hours)</i>
<i>Dequest[®] 2060</i>	<i>0.2-0.4</i>	<i>2.0</i>
<i>Dequest[®] 2051</i>	<i>0.2-0.7</i>	<i>3.0</i>
<i>Dequest[®] 2041</i>	<i>0.3-0.8</i>	<i>4.0</i>

*Sensitivity Limit of the Nuclear Enterprise LMS-1 for
Carbon-14*

We have done no experimental work to determine the sensitivity limit for Carbon-14 on this instrument for analytical scale applications. We think it would be in the range of several hundred to several thousand dpms. It must be recognized that the sensitivity of a radioactivity flow detector is not a fixed value but may vary significantly with the parameters of both the detector and the separations. We can estimate a sensitivity if the values of the parameters are known. Let us first identify the major parameters, assume realistic values and estimate a Carbon-14 sensitivity:

<i>Major Parameters</i>	<i>Assumed Value</i>
<i>1. Radioactivity detector volume</i>	<i>150 μl</i>
<i>2. Eluant flow rate</i>	<i>1.5 ml/min</i>
<i>3. Detector radioactivity background</i>	<i>100 cpm</i>
<i>4. Detector counting efficiency</i>	<i>0.20 cpm/dpm</i>
<i>5. Sample component dilution factor after separation</i>	<i>4.5 ml</i>

From parameters 1 and 2, it follows that the sample residence time in the detector is 0.1 minute

($\frac{150 \mu\text{l}}{1.5 \text{ ml/min} \times 1000 \mu\text{l/ml}} = 0.1 \text{ min}$). Since the background

is 100 cpm the background contribution during 0.1 min. is 10 counts. At a signal/background ratio of 2/1, the most radioactive (center) 150 μ l increment of eluted sample must register 2 X background or 20 net counts/0.1 min.

At a count efficiency of 0.2 cpm/dpm the 20 net counts represent 100 net decompositions/0.1 min. present in the most radioactive 150 μ l increment of eluant. Finally, if one may assume that the eluted radioactivity peak shape is symmetrically triangular, the total radioactivity in the eluted peak is determined by multiplying the radioactivity in the center increment by one half the number of 150 μ l increments in the eluted volume ($100 \times \frac{4.5 \text{ ml} \times 1000 \mu\text{l/ml}}{150 \mu\text{l} \times 2} = 1500 \text{ decompositions/0.1 min} = 15,000 \text{ dpm}$).

The sensitivity of detection of Carbon-14 is thus 15,000 dpm under these parameters. In radiochemical purity analysis at these same parameters, a 1,500,000 dpm sample is required to detect a 1% radioactive impurity. To improve sensitivity one can

- increase detector volume (if component resolution allows)
- decrease background
- decrease eluant flow rate
- increase detector efficiency
- reduce dilution volume

It is also evident that detector sensitivity will increase with higher sample specific activity.

Extensions of Technique to Larger Scale Preparative Purification

We have recently increased the preparative scale from the previous limit of 500 mg sample size to 2000 mg for DEQUEST[®] 2060 and obtained about 98% composition purity. This increase was accomplished primarily by use of the larger column (Pharmacia K 50/60). The resin bed was 5 X 35 cm. The ammonium carbonate eluant was again applied by linear gradient mode over a molarity range of 0.15 to 0.30 in 3 hours at a flow rate of 7.5 ml/min. Other equipment changes included 1) the substitution of a 4 ml sample injection loop made from 1/8 inch OD stainless steel tubing and 2) use of retaining struts on the chromatography column to prevent outward slippage of the adjustable end plugs at the higher pressures from increased flow rates. The impure DEQUEST 2060 sample in this project was not radioactive. To detect the separated sample components under gradient elution conditions, radiolabeled forms of the major sample components were added. This permitted use of the LSM-1 flow detector operating near its maximum sensitivity range.