

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

APPLICATION OF NEW LIQUID SCINTILLATORS
AND 6 ML VIAL
FOR REDUCTION OF
SCINTILLATOR AND RADIOACTIVE WASTE

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Increasing costs of radioactive waste and solvents have made it necessary to apply new approaches in LSC sample preparation. New liquid scintillators are capable of incubating twice as much or even more of aqueous samples than the classical colloidal scintillator systems with a stable and smaller and uniform size micelle formation and without formation of viscous liquid crystals. Counting efficiency remains at the same level as before when using small vials fitting inside of the standard classical ones.

I. INTRODUCTION

In the infancy of the LSC technology the final sample volume exceeded 40 ml. The volume of the vial has since subsequently been standardized to 20 ml for over two decades. Most of the instruments are also built accordingly to measure and collect the counts of the sample in such vials, and until recent the volumes of the liquid scintillator have been usually between 8 and 10 ml. Ever increasing number of LSC samples has been raising the annual costs and physical volume of the scintillators and vials. Since the research funds at the same time have become in short supply, the economical factors had to be taken into account. The first cost reduction by search for inexpensive scintillators generated a wide variety of cocktails and many research groups have created their own. Another cost reduction in the LSC was the introduction of the plastic vial in 1963.

II. LSC WASTE PROBLEM

A liquid scintillation instrument does not only produce counts, but also radioactive waste. If we consider that an average instrument counts 20,000 samples per year using 200 liters of liquid scintillator, it produces more than this as waste. If the instrument uses 10 ml scintillator with 1 ml active sample and processes 20,000 experiments per year, it creates 220 liters of liquid and about 1,500 liters of solid waste from disposable plastic vials. Now the radioactive waste is usually destroyed by combustion, while earlier a considerable part of such aqueous - organic waste was poured into the regular sewage system.

Today the handling of waste is covered in varying extent by governmental regulations in all countries, and is causing considerable waste treatment bills. The costs of disposal of the solid and liquid radioactive waste vary, but in many countries the disposal costs are close to the costs of scintillators. Also controversially today the disposable type of vial represents the majority of costs in the waste treatment.

During this decade commercial products have largely replaced the homemade scintillators due to the fact that the standardized quality of commercial products saves time, guarantees reliable results and yet prices to the user have been kept reasonable.

We would like to refer to the introduction of miniature bags in the 1970 LSC congress in the same place. Many of us were surprised by the reference (Gupta, 1971) to the volume of scintillator and the numbers of measurements made annually worldwide. Within the nine years their numbers have certainly not decreased. The estimated annual volume of solid and liquid waste produced by LSC, based on 20,000 samples per instrument and year for a total of 15,000 instruments, is approximately 25 million liters.

Today samples can be counted with high efficiency in small vials with less reagents. Subsequently the final costs per sample, consisting of vial, reagent and radioactive waste, are reduced. The following figure gives a direct comparison how much miniaturizing for a 1 ml sample, mixed with 10 ml liquid scintillator and reduced into 5 ml scintillator, saves in average costs in some countries.

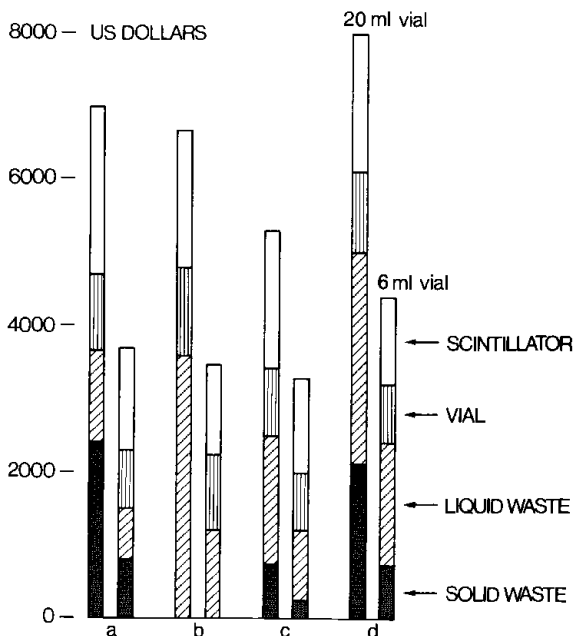


FIGURE 1. Distribution of total annual sample costs per instrument in West Germany (a), France (b), Belgium (c) and in The Netherlands (d).

III. MATERIALS AND METHODS

LSC measurements were performed at 12°C in LKB Ultrabeta 1210 liquid scintillation counter (LKB/Wallac, Turku, Finland) with a ^{226}Ra external standard. All counts were an average of triplicate samples. Cross counts were accumulated till 10.000 cpm.

Liquid scintillators, used in the experiment, were of standard commercial quality, such as Aqualuma^R Plus, Rialuma^R and Lumagel^R (Lumac Systems AG, Basel, Switzerland), and Triton-X 100 toluene scintillators prepared according to Patterson and Green (Patterson, 1965). Scintillation vials were from the same company as the liquid scintillators (Milli^R-6/Milli-20 glass and polyethelene).

Sample holding capacity was estimated visually on apparent homogeneity, and the homogeneous samples were tested further for phase contact with hydrophilic and lipophilic tritium standards by measuring the counting efficiency. The homogeneity was considered established when a scintillator loaded with 10% water gave less than 4% relative difference in cross counts measured as above. Internal push-in glass cup standards (InstandTM, Lumac Systems AG) of 100.000 dpm. \pm 0.2% tritium were used as a source of lipophilic and hydrophilic radioactive substances.

IV. REDUCTION OF SAMPLE SIZE

From 1970 until now no considerable reduction of scintillator volume has occurred because reagents have not been capable of holding aqueous samples in a small volume without a considerable reduction in the counting efficiency and accuracy. A 7 ml polyethylene vial was introduced 1970, but the acceptance of this first small vial has been hampered by the requirement of specific adaptors and that they were more expensive than the classical ones. Furthermore, lack of proper racks made their handling unpractical. Table I. summarizes the requirements suggested for practical miniaturizing of LSC.

TABLE I. Requirements for miniaturizing

A. Liquid scintillator	B. Instrumentation	C. Counting vial
1. High sample holding capacity	1. No specific requirements	1. Good optical geometry
2. Good quench resistance		2. Applicable for all instruments
3. Low viscosity with sample		3. Safe handling systems

A. Liquid Scintillator

1. *Sample Holding Capacity (SHC)*. SHC can be defined as the minimum volume of scintillator required to keep sample in a homogeneous form, suitable for liquid scintillation counting. Miniaturizing requires considerable sample holding capacity for various types of biological samples.

2. *Quench Resistance.* The sample holding capacity should be combined with a reasonable counting efficiency in order to maintain a high sample throughput and accuracy.

3. *Low viscosity.* It is desirable that the new type of scintillator will mix easily with the sample in the small vial assuring homogeneity.

The Scintillator System

The use of non-ionic surfactants for emulsifying biological aqueous samples with aromatic scintillation liquid results regularly in a change in the physical appearance of the system when aqueous phase concentration is increased. At concentrations of above 15% of aqueous sample and 30-40% emulsifier, a phase separation occurs resulting in a non-homogeneous sample. At higher aqueous phase concentrations an isotropic liquid crystalline and micellar phase is formed which stability is largely dependent on the viscosity of the system. A high viscosity is required to prevent coalescence of the system. Such a high viscosity influences negatively the homogeneity of the sample preparation in small vials and makes such systems unusable in flow cell detectors. The high viscosity dependence makes such systems also very temperature sensitive. Increasing emulsifier concentration does not enhance the stability and reduces the counting efficiencies. If the temperature range in which the LSC samples are prepared and measured could be more narrow, the stability would be increased and a less complex system be used.

Ionic emulsifiers and stabilizers produce low viscous water-in-oil systems with enhanced stability for temperature and electrolytes. The micelle size of this system is uniform and small which also is demonstrated by high counting efficiency and lower dependence of quenching aqueous phase concentrations. An additional advantage of ionic emulsifiers is their solubilizing property for proteinaceous samples.

The microemulsion structure is considered as spherical droplets with a cross sectional area of around 100 nm.

Such systems consist of a continuous phase (a), inter-phase (c) and dispersed phase (b). In liquid scintillators the systems are water-in-oil whereby the dispersed phase represents the aqueous sample.

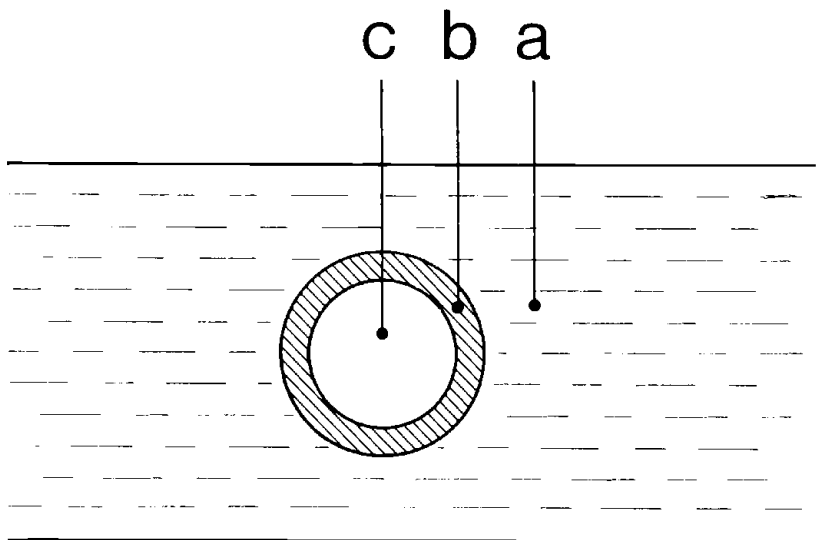


FIGURE 2. Microemulsion system.

Formation of microemulsions are a result of dispersion and stabilization. The dispersion speed is dependent on mechanical mixing forces, but basically on the interphasial tension. If enough surfactant and cosurfactant are present in right proportions and the interphasial tension approaches zero, the phase equilibrium causes a more spontaneous dispersion.

The surfactant system must also be able to stabilize the droplets against coalescence. This requires a sufficient area between dispersed and continuous phase. In the interphasial range there is at least a small (100 cal/mole) binding energy between the surfactant molecules to provide the stabilization. However, it is assumed that each of the surfactants molecules acts independently in the dispersion stabilization process. No stoichiometric relation is needed, but ratios of each emulsifier are important or even critical.

A comparison of the sample holding capacity between the classical and the miniaturized systems is shown in the following phase diagrams. The two upper ones show a new and the two below a typical classical system. Compatibility of phosphate-buffered saline (0.01 M - 0.5 M) in a liquid scintillator is shown as a function of concentration and counting

temperature. Homogeneity is observed during 48 hours each temperature and checked by comparing of counting efficiencies for lipophilic and hydrophilic tritium (^3H -palmitic acid and ^3H -glucose). A homogeneous sample gives an equal counting efficiency for both types of radioactivity.

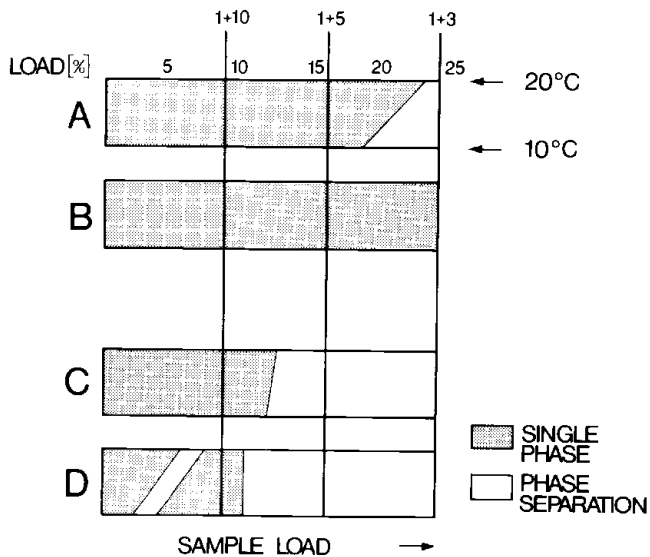


FIGURE 3. Sample holding capacity versus temperature for new (a, b) and classical (c, d) scintillators.

Additional data is shown in the following graphs which illustrate the counting efficiency as a function of the sample holding capacity. Note that the vertical lines indicate scintillator - sample ratio of 10 + 1, 5 + 1 and 3 + 1 ml, respectively.

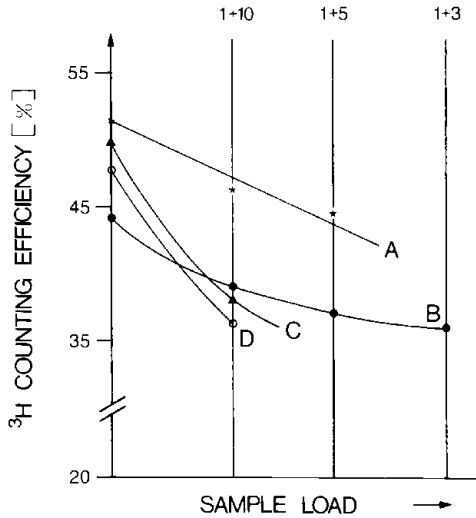


FIGURE 4. Comparison of tritium counting efficiency versus sample load for new (a, b) and classical (c, d) scintillators.

Further miniaturizing under half of the present volume is possible (b), but only with somewhat lower counting efficiencies. Classical products (c and d) show less quench resistance and sample holding capacity.

B. Instrumentation

The change from the classical vial into the small one has been slow although instrumentation for small vials was introduced a few years ago. An optimal solution is obviously an instrument that can use both small and large vials. Since this causes too big a problem in the counting chamber and vial transport systems, it has been easier to design the small vial adaptable to standard instruments by means of using the classical large vial as adaptor.

C. Vial

1. *Optical Geometry.* In addition to the reduction of the scintillator volume to one half or less with the miniaturizing, the narrow diameter of the small vial allows the use of proper height of liquid column for optimizing the geometry and counting efficiency. Since the diameter of the detector in the instruments is about five centimeters, the level of scintillator in the vial influences the overall counting efficiency and quench correction systems.

The corresponding liquid volumes in the classical 20 ml vial and the 6 ml vial are shown in the following graph.

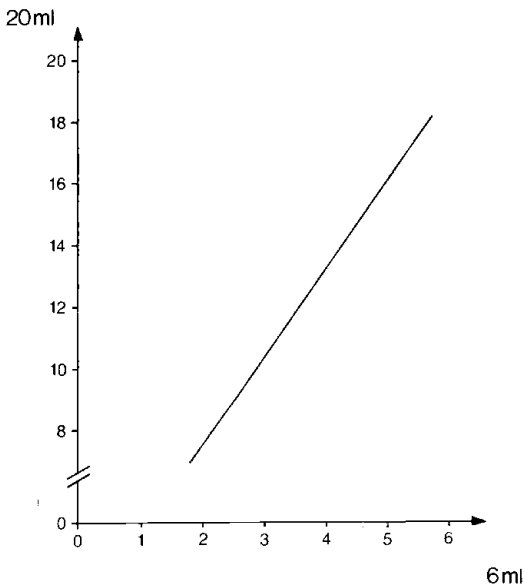


FIGURE 5. Liquid volume comparison for 6^a. and 20 ml polyethylene vials.

a. Inside a standard 20 ml glass vial.

In the following we can see the direct influence of the height of liquid column to cross counts and the volume of scintillation liquid.

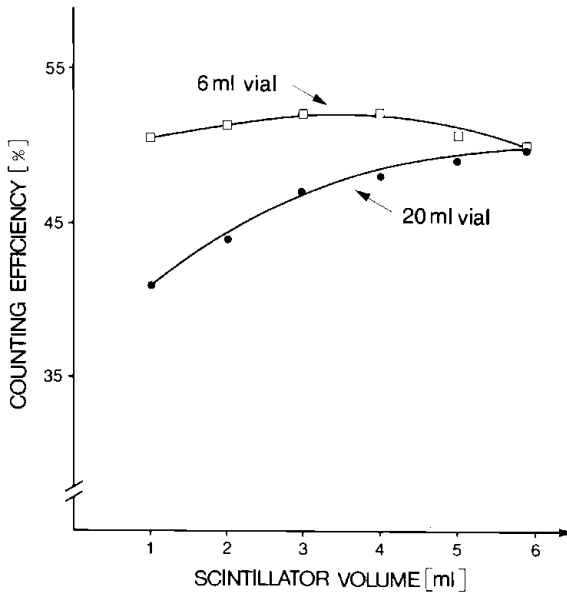


FIGURE 6. Counting efficiency versus scintillator volume in 6 and 20 ml vials.

The effect of volume is partially corrected by the quench correction, but there are limits to the maximum and minimum volume using one and the same corrector curve for both classical and miniature vial types. The range of volume is dependent on the quench correction method and on the instrument type. In this respect it should be remembered that quench correction is different for each individual instrument, vial and type of scintillator, and that the quench correction parameters must not be extrapolated for extreme volumes. The differences in the quench correction between a plastic and a glass vial are similar for both standard and small vials.

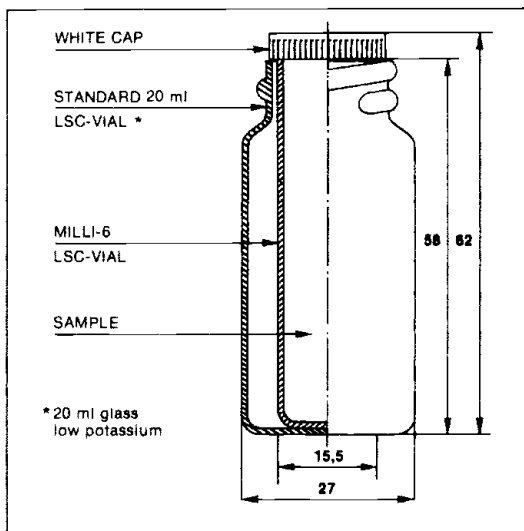


FIGURE 7. A 6 ml vial inside a 20 ml vial.

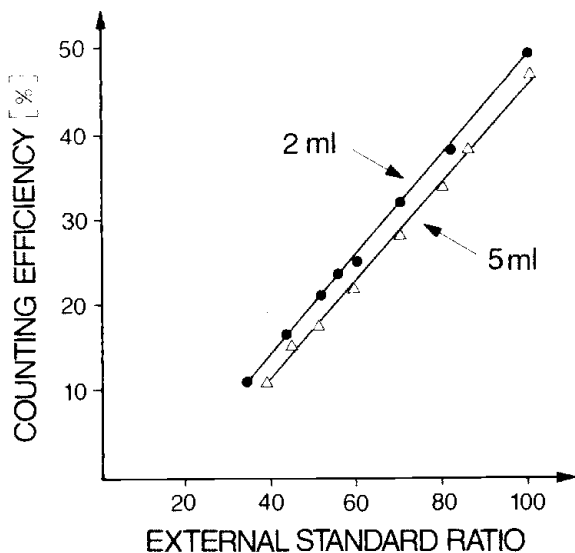


FIGURE 8. Typical volume dependence of external ratio and counting efficiency for ^3H for miniature vials.

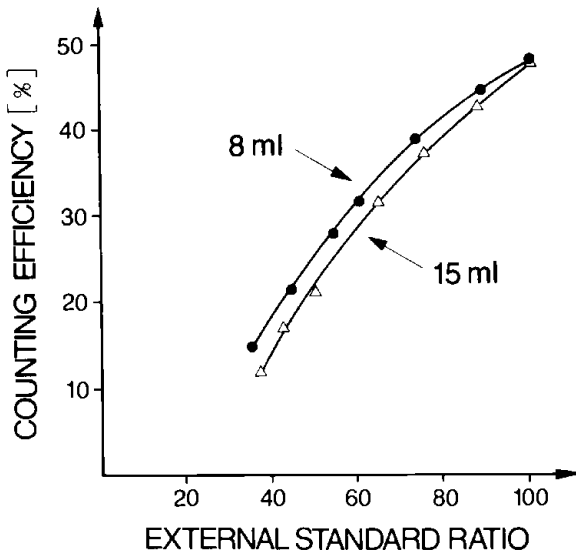


FIGURE 9. Typical volume dependence of external ratio and counting efficiency for ^3H for classical vials.

2. *Applicability.* Ideally a small vial should be compatible for small counters as well as counters designed for 20 ml vials. If a miniature vial can be inserted into a standard counter by using a 20 ml vial as an adaptor, its use is simple.

The adaptor vial has a great influence on the background. For example, the combination of the 6 ml plastic vial inside a low potassium glass vial has a background, dependent on the instrument, of about 24 to 25 cpm. in a tritium channel. The vial combinations may also influence the counting efficiency as is seen in the following picture.

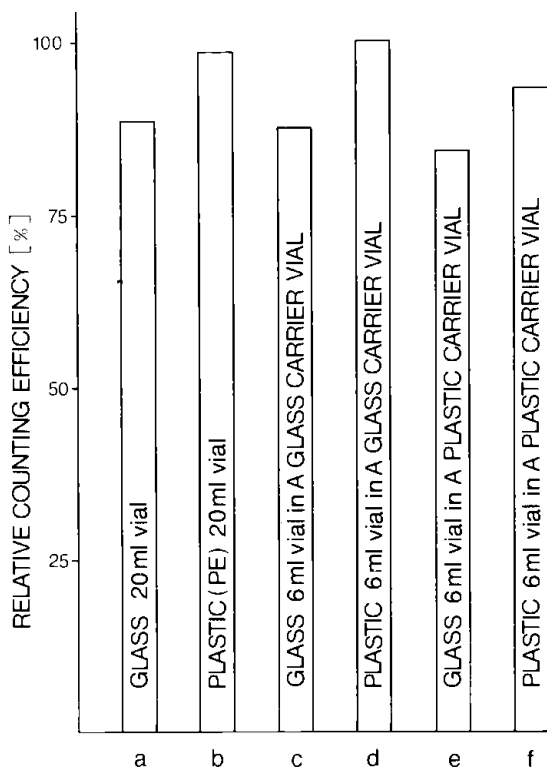


FIGURE 10. Counting efficiencies for classical vials (a,b) and for different vial combinations (c, d, e, f).

3. *Handling.* It is worth mentioning that the storage volume of the small vial is about three times less than that of the classical one, the space requirement in use about one half if appropriate handling systems are used. Because of the small size, handling of smaller vials during transport and pipetting is safest when in a proper carrier.

In certain countries the regulative authorities require separation of solid and liquid radioactive waste. Until recently this separation has been done manually, but now there are mechanical devices on purpose.

V. GENERAL CONSIDERATIONS

With the exception of large volume of low activity sample, the 6 ml vial offers several features over the classical 20 ml systems. These are:

- lower sample preparation costs due to less scintillator;
- considerable lower waste costs;
- equal or higher counting efficiency;
- less space required for storage of reagents, vial, samples and waste.

Provided that good vial systems and high performance scintillators are used, the quench correction limits and accuracy are similar to the classical system, since each new type of vial and new scintillator as well as each instrument requires its own individual standardization.

With the modern instruments it is easy to count an accurate absolute activity due to the data handling capability of microprocessors in the instruments. However, even the best instrument requires a correctly prepared sample.

VI. FUTURE DEVELOPMENTS

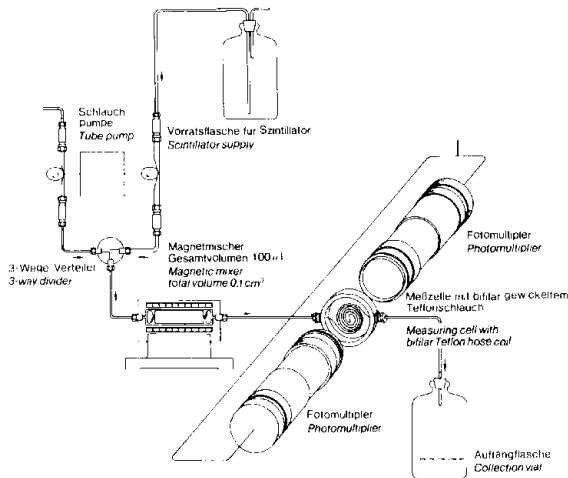


FIGURE 11. Diagram of a flow cell detector system for liquid radiochromatography.

It is predictable that there will be further reduction of liquid scintillator volume with more efficient scintillators and solvent-emulsifier systems. Undergoing development of liquid chromatography systems will automatically lead to an increased use of flow cell technique and measurements of radioactive samples without vials.

REFERENCES

- Gupta, G.N. (1971). "Organic Scintillators and Liquid Scintillation Counting." P. 747 - 752. Academic Press.
- Patterson, M.S., and Greene, R.C. (1965). *Anal Chem.* 37, 854.