

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

INTRODUCTORY REMARKS ON SAMPLE
PREPARATION METHODS

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I. INTRODUCTION

Liquid scintillation counting is certainly the most versatile and most frequently applied technique for quantitative determination of radioactivity in inorganic, organic and biological material. This technique can be used not only for counting low and high energetic beta-particles but also for alpha and gamma-ray emitters. The main application of liquid scintillation counters however lies in the detection of low energy beta-particles from radioisotopes such as tritium, carbon-14, or sulfur-35, which by other methods (Geiger-Muller or proportional counters) may be measured only with much lower efficiency and accuracy.

Due to the basic principle of liquid scintillation counting it is necessary to bring the radioisotope to be assayed in close or intimate contact with the scintillator molecules of the scintillation solution, resulting in either homogeneous or heterogeneous counting samples. The enormous variety of specimen especially those of biological origin, and the different physico-chemical properties of the tracer compounds require appropriate and reproducible methods of sample preparation.

II. PREPARATION OF HOMOGENEOUS SAMPLES

For homogeneous sample counting the radioactive material must be soluble in the organic scintillation solvent (toluene, xylene, dioxane). Unfortunately most inorganic salts, hydrophilic substances, macromolecules (such as proteins, nucleic acid or polysaccharides) or biological tissues (muscle, bone, liver, brain) and body fluids (blood, plasma, urine, spinal fluid) are incompatible with the solubility characteristics of the liquid scintillant. To overcome these problems various useful methods for tissue preparation have been developed such as: solubilisation by hydrolysis, wet oxidation, combustion.

For tissue *solubilisation* sodium or potassium hydroxide, formamide, or especially quaternary ammonium bases are quite useful and are commercially available under the trade names: Hyamine, Soluene, NCS, Protosol, Digestin, Biosolve, Eastman Tissue Solubilizer, etc. In general, these commercial solubilizers have high solution power for many biological tissues but may be inefficient in solubilizing bone, cartilage and collagenous material. Sometimes higher temperatures and mechanical agitation are required. Under certain temperatures the use of solubilizers can cause severe coloration of the sample with consequently high quenching effects. In those cases bleaching of the tissue digest with benzoyl or hydrogen peroxide is recommended but may lead to excessive and long lasting chemiluminescent reactions.

For *wet oxidation* of biological material reagents such as nitric acid, hydrogen peroxide, perchloric acid, or potassium persulfate have been successfully used and possess certain experimental advantages. In general, methods of wet oxidation can be performed directly in the counting vial and do not require any expensive or special equipment. For highly colored specimen (liver tissue, blood, plant leaves) and for those tissue resistant to liquid solubilizers the wet oxidation technique is widely applicable and reliable.

Certainly, the best method of sample preparation is the so called *combustion* technique, which was derived from the Schöninger oxygen-flask method, and has been modified and improved in many ways. Besides manual procedures there are now semiautomated and fully-automated oxidizer models commercially available. In an oxygen atmosphere biological specimens labelled with ^3H , ^{14}C , or ^{35}S can be combusted to tritiated water, ^{14}CO , or $^{35}\text{SO}_2$ which are directly dissolved into the scintillation solution. By this method chemical or color quenching as well as disturbing chemiluminescence reactions are avoided or significantly reduced. Depending on the selected combustion technique solid, dry, wet or liquid samples may be prepared for liquid scintillation counting with high recovery. The various commercially available oxidizer or combustion instruments differ not only in technology and their degree of

automation but also in sample size, number and capacity.

III. PREPARATION OF HETEROGENEOUS SAMPLES

In contrast to the above mentioned methods of preparing homogeneous samples, in heterogeneous sample counting the radio-labelled specimen is not dissolved in the scintillation solvent but finely dispersed or suspended or on a solid support. Accordingly the techniques are differentiated as: emulsion counting, suspension counting, and counting on solid support.

In the past 10 years much research has been done on *emulsion* scintillators and several emulsion cocktails have been developed using various types of special emulsifiers and organic solvents. These water-in-oil emulsions possess high counting efficiency and high capacity for dissolving water and aqueous samples. They have a pronounced solubilizing power for ionic substances and even macromolecules. The efficiency of such an emulsion cocktail depends upon the diameter or thickness of the aqueous micellar phase, the amount of water, and the isotope energy. A very fine dispersion is an ideal colloid and may behave as a true solution. Nevertheless all emulsion counting systems are complex and thermodynamically unstable. The stability and counting efficiency is influenced by factors such as: chemical nature of the sample and its concentration, electrolyte content, pH value, fluor concentration, temperature, agitation, cooling time, and phase composition. Within a certain sample range and under proper handling emulsion cocktails can be successfully applied for a wide variety of samples of different nature. They are available of unrevealed composition under trade names of Insta-gel, Aquasol, Oxifluor, Dimilume, Scintisol, Scintigel, Unisolve, Handifluor, Corusolve, Lumagel, and others.

Another method to prepare insoluble material for liquid scintillation counting is the *suspension counting* technique. Depending on particle size finely ground powders can be measured in suspension by addition of gelifying or thixotropic agents to prevent sedimentation. Scintillation solutions containing certain amounts of substances such as: Cab-O-Sil, aluminum stearate, di-isocyanate, poly-olefin resins, hydroxypropyl methylcellulose or many others, will form relatively stable thixotropic gels, which have been used especially for counting silica gel or other adsorbents from thin-layer chromatography by suspension counting technique. The determination of carbon-14 labelled $\text{Ba}^{14}\text{CO}_3$ has always been a problem, but can be successfully performed in suspension cocktails. However, the counting efficiency is strongly influenced by the

particle size of the carbonate due to self-absorption.

Self-absorption is also the main problem and limiting factor of the method of *counting on solid support*. Radioactive material placed on or filtered through membrane-, paper-, or glassfiber-filter discs may be immersed and counted directly in an adequate scintillator solution. The counting efficiency of this technique depends on the situation whether the radiolabeled compounds remain on the solid support, or be partially eluted by or completely dissolved in the scintillation solution. Besides these factors, the accuracy and reproducibility of solid support and suspension counting is influenced by limitations to maintain a precise and optimum source geometry of the complete counting system.

As we have seen there are several ways and methods for sample preparation for radioactivity measurements by liquid scintillation counting. Depending on the isotope and on the physico-chemical properties of the labelled compounds, as well as on the available laboratory equipment it is necessary to select and adjust the most suitable sample preparation method for each analytical problem. Further methodological research will certainly provide more and better techniques. But still each user of liquid scintillation procedures has to innovate and optimize his specific sample preparation method.