TISSUE SOLUBILIZATION

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The problem being considered here is that of finding or devising chemical reagents and methods of application which will allow the solubilization or digestion of plant and animal tissues so that they can be successfully incorporated into liquid scintillation counting mixtures. The tissue may be whole, homogenized, macerated, or in some other state of subdivision. The methods employed should result, ideally, in digested samples which when added to the scintillation mixtures yield clear, colorless, uniform liquids, exhibiting a minimum of quench, a minimum of chemiluminescence, and a maximum of counting stability. All reagents should be inexpensive, easy to handle, and relatively nonhazardous. Reagents should be capable of digesting large size samples, should be capable of rapid and complete digestion, and should not require great expertise in use. Reagents should be as versatile as possible with respect to the types of tissues for which they can be used, and methods of digestion should allow accurate determination of radionuclide content with minimal systematic error. Finally, although it is not possible to achieve all these desirable characteristics with any given reagent or method, it is possible in many cases to design a reagent method which will be optimum for a given sample and set of experimental conditions. Some examples of solubilizing agents and procedures for their use will be given in the following text.

Methanolic Potassium Hydroxide (1)

This reagent was among the first used for solubilization of whole animal tissues. It has the advantages of low cost and rapid digestion for a variety of animal tissues; however, it suffers the disadvantages of limitation of sample size, low counting efficiencies (particularly for tritium), has a potassium-40 background which may prove a problem for low activity samples, and may not always completely digest a sample.

Colorless, Concentrated Nitric Acid (2)

In general, this reagent has the same low cost and rapid digestion advantages and the same low sample capacity and low counting efficiency disadvantages as potassium hydroxide. Examples of tissues it has been used to digest are shaved rat skin (2), and rat brain, skeletal muscle, stomach, liver, and spleen (12).

Formamide (3)

A variety of tissue types have been digested with this reagent including animal epidermis. It has the property of being a strong tissue solubilizer; however, it also is a strong scintillation quencher. It may prove advantageous to use where a sample combines difficulty of digestion and a high-energy nuclide to be assayed.

Sodium Hydroxide Solution (4)

In the reference cited above, the investigator reported that he found it necessary to add Cab-O-Sil to his sample to prevent a constant decrease in counts over a period of ten hours. Stability may be a problem when using this reagent. In general, sodium hydroxide is a less desirable digestion agent than potassium hydroxide because of the reduced solubility of sodium salts compared with potassium salts.

Quaternary Hydroxides and Chlorides

Many references exist on the use of these types of solubilizers. The general method for digesting a sample is to mince, grind, or macerate the wet, whole tissue in a vial. The solubilizer is added and the mixture is digested at a moderately elevated temperature until the sample appears homogeneous. This procedure may take up to several days for solid tissue samples. The next step (optional) is to partially neutralize the digested sample before the scintillator is added. This usually gives higher counting efficiencies, lower backgrounds and improved sample clarity. The disadvantages of these types of solubilizers are slow digestion, high cost, and color-producing reactions with some scintillators. Advantages are relatively high counting efficiencies, and large sample capacity. In general, most basic solubilizers tend to produce chemiluminescence. Quaternaries are particularly prone to this problem. Many times acidification of the digest will reduce or eliminate this phenomenon. When compared with other solubilizers, the quaternaries offer the most versatility with respect to the variety of tissue types which they can be used to solubilize.

However, their high cost and other disadvantages must also be considered if an optimal choice of solubilizer is to be made for given sample type. Although quaternaries are used primarily for animal tissue solubilization, they can be used with soft plant tissue (6). They are unsuitable, however, for digesting the hard parts of plants which remain undissolved even after long digestion periods. Examples of commercial quaternary solubilizers are Hyamine-10-X (Rohm and Haas), Protosol (New England Nuclear), NCS (Amersham/Searle), and Soluene (Packard).

Perchloric Acid and Hydrogen Peroxide (5)

Protein was digested and the heme completely decolorized by this combination of reagents. A clear solution results which can be incorporated into an appropriate counting solution. This method is claimed to be useful for digesting tissue labelled with a variety of nuclides. One investigator (6) found this reagent gave good results when it was used for digesting dried and hard plant material. Another investigator (9) using this reagent digested rat tissue labelled with transuranium elements.

Concentrated Nitric Acid, Perchloric Acid, and Magnesium Nitrate (7)

This reagent can be used for oxidizing sulfur-35 labelled tissue to magnesium sulfate. The oxidation products are then dissolved in glycerol and the mixture diluted with ethyl alcohol and N,N-dimethylformamide. A modification (6) of this reagent gave good results for routine analyses of dried and hard plant material.

Nitric Acid and Perchloric Acid (8)

This mixture was used to digest a variety of plant and animal tissues. Also it is claimed that it can be used for sample preparation of tissues labelled with a variety of radionuclides.

Sodium Hydroxide, Distilled Water, Methanol and Triton X405 (10)

Mammalian tissue was digested with this reagent, neutralized with nitric acid, and counted in a toluene/triton scintillator.

Sodium Hydroxide and Bio-Solv BBS-2 (11)

This combination of reagents is reported to give rapid solubilization of solid animal tissue. The tissue is pretreated with 1 N sodium hydroxide and then neutralized with

the BBS-2 solution. Although the sample size is limited for this method, relatively high efficiencies are attainable and the reagents are inexpensive. Bio-Solv BBS-2 is a Beckman Corp. product.

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