

ROUND-TABLE ON  
'PREPARATION OF THE ALKALINE ABSORBENT  
FOR RADIOACTIVE CO<sub>2</sub> IN LIQUID  
SCINTILLATION COUNTING'

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OUR problem was the counting of respiratory CO<sub>2</sub> produced in metabolic studies on small animals that had received C<sup>14</sup> labeled substrates. Since in this type of experiment there are usually large numbers of samples to be assayed, liquid scintillation counting offered the maximum economy in time and, more important, the maximum sensitivity, at least compared to anything we were using.

PASSMAN *et al.*<sup>1</sup> had described the preparation of an organic base suitable as an absorbent for CO<sub>2</sub>. We followed their procedure accurately and invariably ended up with a solution of 'Hyamine' base that either would not clear or which, if clear, gradually turned color with time. In either case the solution was unsatisfactory for liquid scintillation counting.

In view of our failure we turned to an ion exchange method for the preparation of the base. Dowex 2, a strong base anion exchange resin, is converted from the chloride to the hydroxide form and back again repeatedly until the washings between each cycle are colorless. It is finally converted to the hydroxide form. Ten equivalents of resin for each equivalent of 'Hyamine' to be exchanged are placed in a column with sufficient space above the resin to allow for expansion of the resin. The resin is then eluted exhaustively with methanol to remove water and the elution continued until the eluate is colorless. The methanol is then removed by exhaustive elution with toluene. A saturated solution of recrystallized 'Hyamine' in toluene is poured on the resin and washed in with toluene. Collection of the eluate is begun at the appearance of alkalinity and is continued until the alkalinity begins to fall off. The alkalinity of the eluate is followed by acid titration. The concentration of the resulting colorless toluene solution is 0.1–0.2 M. The product is stable in the cold indefinitely but slowly becomes colored at room temperature. Attempts to concentrate this solution were unsuccessful owing to its thermolability, so that its usefulness is limited by its low concentration. Thus we found that it was not suitable for counting weakly radioactive CO<sub>2</sub>, where to get sufficient counting accuracy large amounts of CO<sub>2</sub>, and therefore large amounts of

base, would have to be used making any diffusion method technically unfeasible. It is useful, however, for counting other acidic compounds such as amino acids, proteins, purines, and sugar acids which can simply be dissolved in the solution. Furthermore, any organic compound that can not be handled intact can be burned to  $\text{CO}_2$  and counted in the base.

The problem of counting weakly radioactive  $\text{CO}_2$  remained and we returned to the method of PASSMAN *et al.*<sup>1</sup> and after making appropriate modifications in the procedure we were able consistently to prepare a stable colorless product.

The modified method is as follows: 96 g (200 mmole, M.W. 480) of 'Hyamine' recrystallized from 4 volumes of toluene, 1.8 ml (100 mmole) of  $\text{H}_2\text{O}$ , and 100 ml of methanol are combined in a dark glass-stoppered bottle. All solvents must be reagent grade; in our experience of several brands tested, only the toluene supplied by Fisher or Merck was satisfactory. The solution prepared above becomes cold and must be warmed to room temperature before the next step. The final volume of this solution is about 200 ml which will ultimately yield a product about 1 M in concentration. Working at this concentration rather than in more dilute solution eliminated the need for later evaporation which we found to be a trouble spot in the procedure cited. 25.5 g (110 mmole) of Fisher  $\text{Ag}_2\text{O}$  are added to the solution and shaken violently for 10 min. The time is critical as any longer reaction time in our experience will lead to an unsatisfactory product. The suspension is then quickly centrifuged and the residue of  $\text{AgCl}$  and excess  $\text{Ag}_2\text{O}$  is discarded. The supernatant fluid may be slightly turbid but is ready for the next step which consists of illumination of the solution to get rid of silver compounds according to the procedure of PASSMAN *et al.*<sup>1</sup> At first we illuminated the solution artificially but more recently we simply expose it to sunlight in a clear glass-stoppered bottle. After a day a sediment appears and the solution is again centrifuged. The supernatant fluid at this point is yellow and clear. After a day or two more of the illumination the now turbid fluid is again centrifuged to yield a clear colorless product varying in concentration from 0.8–1.0 M and stable indefinitely at room temperature. We have had some of this material on hand for 10 months during which time there has been no change in appearance, titer, or counting efficiency when tested with a standard sample of  $\text{C}^{14}\text{O}_2$ . We keep the solution in a flask and it is dispensed through an all glass siphon. There is no danger of the stopcock 'freezing' nor is any lubricant needed since this solution does not etch glass and by its viscosity serves as its own lubricant.

In the application of this solution to  $\text{CO}_2$  counting we use the diffusion flask shown in Fig. 1. Three ml of the base are placed in the center well. The sample to be assayed is placed in the outer chamber and may contain up to 2 mmole of  $\text{CO}_2$  in the form of a water soluble or insoluble carbonate. Water is added to this chamber if necessary to make the volume of liquid 15–20 ml. Three ml of 1 M  $\text{HClO}_4$  are measured into the side arm from a hypodermic syringe

fitted with a flexible silver cannula. The stopper is greased, inserted, and held secure by a heavy rubber band around the hooks. The acid is then tipped in. The flask is shaken for 1hr as rapidly as is possible without splashing on a reciprocating shaker. A shaker supplied by the Arthur H. Thomas Co. has provision for 15 such 250 ml flasks. At the end of the hour 2 ml of the base

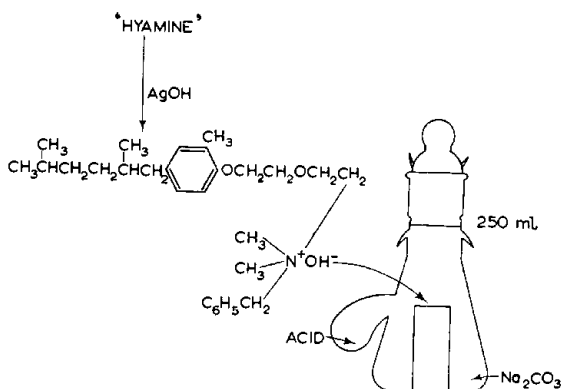


Fig. 1.

are withdrawn from the center well and added to 14 ml of toluene containing 2,5-diphenyloxazole (PPO) at a final concentration of 0.4%. In our experience the best PPO is supplied by Pilot Chemicals, Inc., Waltham, Mass. The counting efficiency for this system is 40%.

REFERENCE

<sup>1</sup> PASSMAN, RADIN and COOPER. *Anal. Chem.*, **28**, 484, (1956).



PART IV

GENERAL APPLICATIONS

