

Chapter 24

Liquid Scintillation Counting of $^{14}\text{CO}_2$ as $\text{Na}_2^{14}\text{CO}_3$

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INTRODUCTION

A large variety of techniques, absorbents and scintillants have been proposed for the radioassay of $^{14}\text{CO}_2$. The methods cited fall mainly into two groups, those used for the estimation of the relatively small amounts of $^{14}\text{CO}_2$ released during Warburg assay and others dealing with the handling of the large quantities produced during combustion of biological material.

Certain organic agents commonly used for trapping $^{14}\text{CO}_2$ have been reported to cause gross inhibition of enzyme activity if present in closed systems and for this reason Duncombe *et al.*¹ recommended the use of aqueous KOH for $^{14}\text{CO}_2$ liberated as the result of enzyme activity. Their method of assay however was based on an internal standard gel suspension system and as such did not take advantage of modern methods of estimating counting efficiency such as sample channels ratio (SCR) or external standard channels ratio (ESCR) techniques. Overcoming the disadvantages of the higher background associated with KOH, Murray² used NaOH as the trapping agent which was accommodated in a toluene/Triton X 100 scintillant. Excellent recoveries were obtained and activities could be measured by SCR or ESCR methods but the scintillant had a limited capacity for NaOH and the alkali-induced chemiluminescence necessitated an overnight holding period before assay.

During a survey of proprietary commercially available liquid scintillants it was noted that a micellar scintillant, NE 260, was exceptional in its ability to handle high concentrations of NaOH and Na_2CO_3 whilst retaining the appearance of a single phase counting system. This scintillant, the formulation of which is based on work communicated at a previous symposium,³ has been examined as a possible vehicle for the assay of $^{14}\text{CO}_2$, as $\text{Na}_2^{14}\text{CO}_3$, and some aspects of this work are now reported.

EXPERIMENTAL

Materials

Sodium carbonate [^{14}C] 57 mCi mmol⁻¹ was purchased from the Radiochemical Centre, Amersham, Bucks, and depending on requirements either diluted with water or added to carrier Na_2CO_3 to give a required specific activity. NE 260 scintillant was obtained from Nuclear Enterprises, Edinburgh, and glass and polythene vials from Koch Light, Colnbrook. For some experiments vial caps were modified by drilling a 2 mm hole through the cap and replacing the tin foil/cork lining with a rubber septum. Small inserts varying in volume from 1-5 ml were machined from PTFE and used to restrict the volume of the head space above the liquid scintillator.

Counting was done routinely using an Isocap (Nuclear Chicago) machine at ambient temperature and comparison made, in the $^{14}\text{CO}_2$ recovery experiment only, with data obtained using a Model 3385 (Packard) spectrometer operated at 10 °C. Estimates of

activity were carried out using SCR and ESCR techniques employing a set of commercially available quenched standards.

Methodology

Where various levels of carrier Na_2CO_3 and NaOH were required in particular experiments these were dispensed to the vial first by addition of the calculated volumes of solutions of the requisite molarities. After addition of active sample the volume of the aqueous phase was made up to 1.2 ml with water. It should be noted that this practice has been followed throughout this work and any volumes or amounts of $\text{Na}_2^{14}\text{CO}_3$ or NaOH stated as being added to vials were always made up to a total aqueous volume of 1.2 ml before addition of 10 ml NE 260 scintillant. The vials were then shaken vigorously by hand three or four times until a clear final mixture was obtained. It is important not to leave scintillant in contact with strong NaOH for prolonged periods before shaking otherwise a cloudy final counting system may result. Activity was usually estimated after a dark adaptation period of 2 h but identical results are obtained after a holding period of 24 h provided carrier Na_2CO_3 and NaOH are present at minimum recommended levels.

Activity in the head space of vials was determined by removing a 7 ml gas volume via the modified cap using a hypodermic gas-tight syringe. The gas was slowly bubbled into 1.2 ml of a solution of 0.4M Na_2CO_3 containing 0.6M NaOH before addition of scintillant. In studies concerned with the determination of the distribution coefficient K , variation in the volume of head space was brought about by introduction of the PTFE inserts (see Fig. 4).

Estimation of activity adsorbed on vial walls was made after discarding vial contents and inverting for a standard drainage time of 30 s. A carrier solution, identical to that used for trapping $^{14}\text{CO}_2$ activity from head space samples, was allowed to completely wet the vial walls before scintillant was added. The efficacy of a strong NaOH solution to trap $^{14}\text{CO}_2$ and the ability of the scintillant to incorporate and count such a solution was tested in the following manner. Amounts of Na_2CO_3 ranging from 4.0-9.3 mmol were weighed into test tubes and 2.0 ml 0.05M Na_2CO_3 containing approximately 0.4 μCi activity added. The tubes were immediately placed within 500 ml B24/29 round bottomed flasks containing 10 ml 5M NaOH, selected such that the rim of the test tube sat loosely within the ground glass neck of the flask which was sealed by a rubber serum cap. Release of $^{14}\text{CO}_2$ was accomplished using 9M H_2SO_4 and after holding for 45 min, with occasional shaking to aid the trapping of $^{14}\text{CO}_2$, 1.0 ml portions of the NaOH solutions were removed to counting vials containing 0.2 ml M Na_2CO_3 . After a dark adaptation period of 2 h activity was estimated at regular intervals during the following 22 h, both spectrometers being used.

RESULTS

Initial experimentation established that 10 ml of the scintillant could accommodate up to 5 mmol NaOH and at least 600 μmol Na_2CO_3 while retaining the appearance of a single phase system.

In examining the counting characteristics of mixtures of scintillant and aqueous $\text{Na}_2^{14}\text{CO}_3$ in the absence of NaOH it was observed that the measured activities of dilute solutions were less than expected. Figure 1 illustrates the loss of activity noted when portions of 0.02M $\text{Na}_2^{14}\text{CO}_3$ containing approximately 0.2 μCi ml⁻¹ were assayed in glass vials. The SCR method was used to calculate dpm values which were obtained from vials counted 30 min after preparation and further losses were apparent after extended holding times. The theoretical activity value was taken as that measured as above in the presence of carrier Na_2CO_3 and NaOH.

The apparent increasing loss of activity with decreasing carbonate concentration suggested that adsorption was occurring within the vial even though the specific activity of the carbonate used was much less than that suggested as necessary for the occurrence of this effect with organic compounds. This facet was examined more closely using carbonate solutions of similar total activities but differing specific activities and comparing these with hexadecane [^{14}C] samples quenched with chloroform, NE 260 being used as scintillant in all vials. Results are shown in Fig. 2.

As expected the values obtained from samples containing added carrier lay very close to the line established using the hexadecane samples but significant deviations were

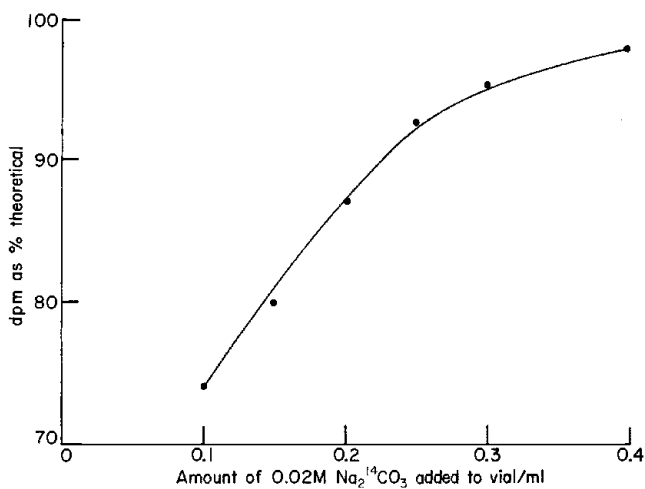


Fig. 1. Variation in percentage activity found with volume of $0.02\text{M Na}_2^{14}\text{CO}_3$.

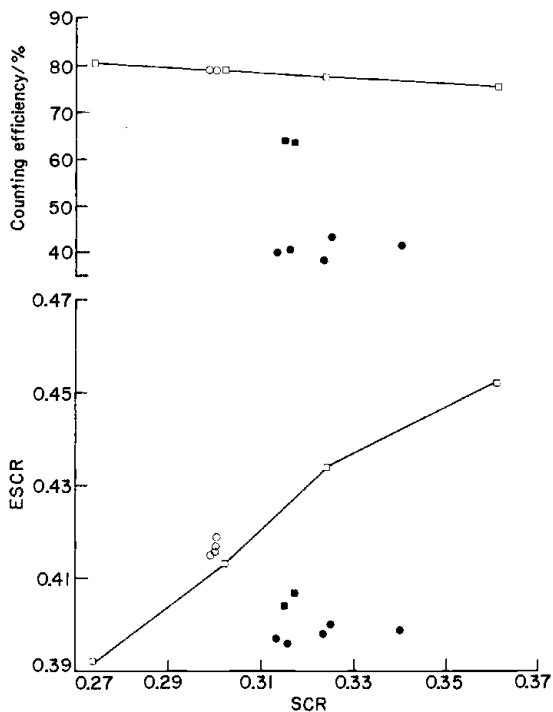


Table 1. Retention of $\text{Na}_2^{14}\text{CO}_3$ activity, as determined by SCR technique, by glass and polythene vials.

Vial no.	Type ^a	Contents	Initial ^b dpm	dpm after ^b refill
1	G	0.2 ml 5×10^{-6} M $\text{Na}_2^{14}\text{CO}_3$	61.9	9.6
2	G	0.2 ml 5×10^{-6} M $\text{Na}_2^{14}\text{CO}_3$	60.0	17.0
3	P	0.2 ml 5×10^{-6} M $\text{Na}_2^{14}\text{CO}_3$	73.5	6.9
4	P	0.2 ml 5×10^{-6} M $\text{Na}_2^{14}\text{CO}_3$	67.9	7.0
5	G	0.2 ml 2×10^{-2} M $\text{Na}_2^{14}\text{CO}_3$	69.3	12.4
6	G	0.2 ml 2×10^{-2} M $\text{Na}_2^{14}\text{CO}_3$	70.0	13.1
7	P	0.2 ml 2×10^{-2} M $\text{Na}_2^{14}\text{CO}_3$	82.7	13.0
8	P	0.2 ml 2×10^{-2} M $\text{Na}_2^{14}\text{CO}_3$	85.9	6.1
9	G	As vial no 1 + carrier	100.0	2.5
10	G	As vial no 5 + carrier	100.0	2.6

^a G = glass; P = polythene.

^b Expressed as a percentage of the activity found initially in vials 9 or 10.

apparent in the higher specific activity samples, the deviations being mirrored by the dramatic fall in counting efficiency seen in the same samples. The abnormally high SCR values obtained from vials containing high specific activity material strongly suggests a degradation of the ^{14}C energy spectrum due to adsorption on the vial walls. Similar results have been obtained from ^{35}S sulphate samples by Bush, who first suggested the use of such double ratio plots as an indicator of this phenomenon. A feature common to all vials containing low total carbonate levels is that the SCR value soon after preparation, e.g. 0.4126 after 5 min, falls rapidly on holding to a value, e.g. 0.3257 after 1 h, which is of course considerably higher than that obtained from samples containing added carrier. The fall is not due to decay of chemiluminescence and may reflect a change in the extent of adsorption with time. Confirmation that activity is indeed adsorbed by both glass and polythene vials and findings, selected from those obtained in a large number of trials, are given in Table 1. The activity levels after refill in vials 9 and 10 suggest that a maximum of 2.6% of the contents are retained on draining and the higher values obtained from the other vials reflect the extent to which adsorption takes place. In general our results indicate that polythene retains carbonate to a lesser extent than glass although individual results may vary widely.

The loss in count rate on extended holding referred to earlier is illustrated in greater detail in Fig. 3. Approximately 15% of activity is lost from both samples during the assay period but the total loss will almost certainly be greater than this because of the time between preparation and initial assay. The findings suggest that under the conditions indicated in Fig. 3 activity is being lost, presumably to the head space, and such losses will of course be additional to any incurred due to adsorption. These effects in combination explain the fairly large discrepancies noted in the initial activity values quoted in Table 1. Counting blanks prepared and assayed under identical conditions showed negligible activity and eliminate the possibility that the drop in count rate seen in Fig. 3 arises from decay of chemiluminescence. The slow attainment of equilibrium inferred from the gradually falling count rate agrees with the work of Dryden⁸ who, using aqueous K_2CO_3 solutions, noted that the release of CO_2 to a sealed system was effected over a period of hours. Loss to the gas phase was confirmed by head space analysis using modified vials containing solutions identical in composition to those used to obtain data for Fig. 3. Activities found, when adjusted for the fraction of head space sampled, were in reasonably good agreement with counts lost

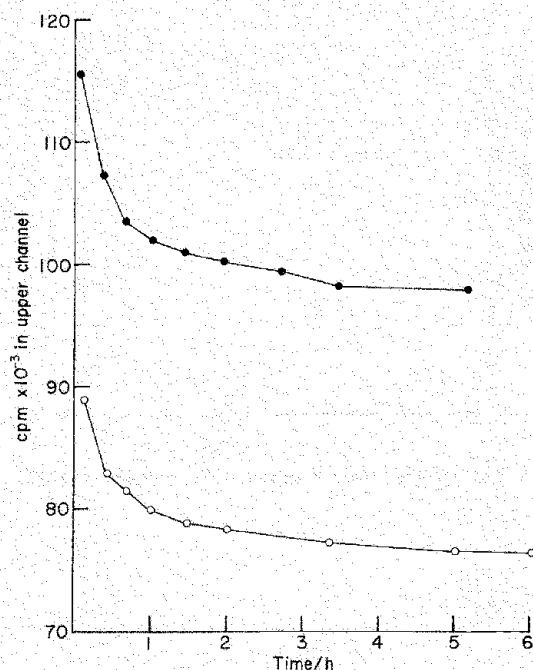


Fig. 4. Decrease in count rate with time in $\text{Na}_2^{14}\text{CO}_3$ solutions. ● = 0.3 ml. 2×10^{-2} M $\text{Na}_2^{14}\text{CO}_3$; ○ = 0.3 ml. 5×10^{-6} M $\text{Na}_2^{14}\text{CO}_3$.

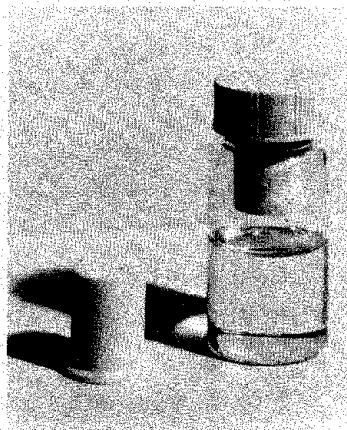


Fig. 5. PTFE inserts used to restrict head space in counting vials.

from such vials. Further investigation of the loss of added activity to the gas phase was carried out using $8 \mu\text{mol Na}_2^{14}\text{CO}_3$; the head space being reduced by the insertion of PTFE inserts (Fig. 5) varying in volume between 1-5 ml. As the head space volume was decreased (total vial volume = 24.9 ml) the measured count rate increased confirming that activity was being distributed between the gas and liquid phases. Horrocks *et al.* in similar studies indicated that a plot of gas phase volume against the reciprocal of count rate should give a straight line from which K , the distribution coefficient, can be calculated. A similar plot of the present results showed that a straight line relationship is obtained (Fig. 6) from which it can be inferred that the radiocarbon is partitioning between the gas and liquid phases according to the simple relationship

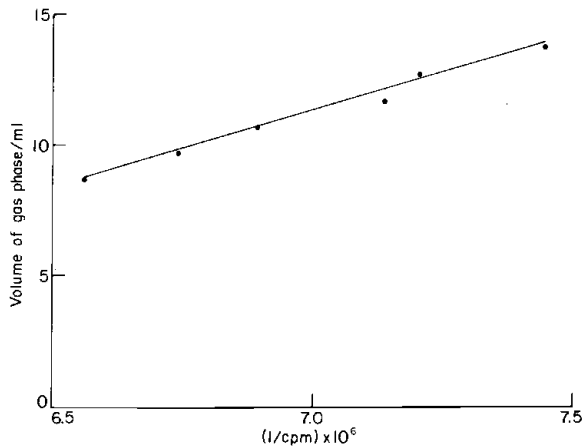


Fig. 5. Plot used for evaluation of distribution coefficient, K . Vials counted 20 h after preparation

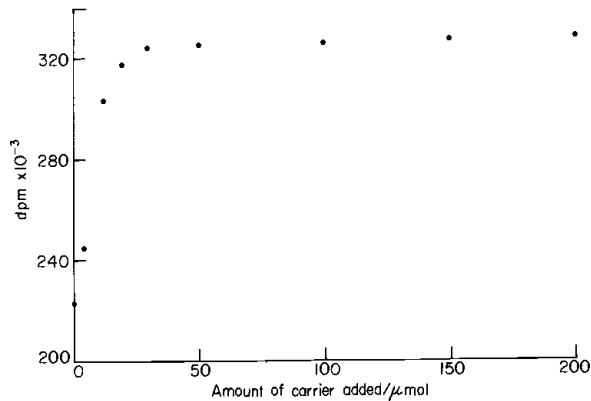


Fig. 6. Effect of increasing carrier concentration on activity as determined by SCR technique.

$$\frac{\text{Concentration } ^{14}\text{C activity in solution}}{\text{Concentration } ^{14}\text{C activity in space}} = K$$

The value of K found here, 2.8, is similar to that found by Horrocks¹⁰ (2.2 at 24 °C) using gaseous $^{14}\text{CO}_2$ and a toluene-only scintillator. Presumably the presence of the additional components in this system partly suppresses the loss of CO_2 to the gas phase.

The effect of adding carrier to a standard 4 μmol $\text{Na}_2^{14}\text{CO}_3$ is shown in Fig. 6. Activity was determined by SCR technique after a holding period of 22 h. Little additional activity can be noted after 30 μmol carrier carbonate have been introduced although amounts of carrier carbonate up to 500 μmol were used. When the volume of gas phase in the samples containing ≥ 34 μmol $\text{Na}_2^{14}\text{CO}_3$ was decreased by insertion of the PTFE inserts no concomitant increase in count rate was noted. Furthermore analysis of head spaces in vials containing 50-150 μmol carrier, i.e. a total of 54-150 μM $\text{Na}_2^{14}\text{CO}_3$, showed that the activity associated with this phase is about 0.5% at the lower level and approximately 0.2% at the higher. The addition of 500 μmol NaOH to vials containing 200 μmol $\text{Na}_2^{14}\text{CO}_3$ reduced the proportion of activity found in the head space to a maximum of 0.07% of the total activity and dpm values estimated by SCR technique

were in excellent agreement with those calculated from the specific activity of the material as supplied by the Radiochemical Centre. It would thus appear that, when the amount of $\text{Na}_2^{14}\text{CO}_3$ exceeds 50 μmol per vial, loss of $^{14}\text{CO}_2$ to the gas phase is very low and addition of $^{14}\text{NaOH}$ decreases this loss to insignificant proportions. Titration of 10 ml scintillant after addition of 0.5 ml water using BDH 6676 indicator showed that 15 μmol NaOH were required to raise the pH to 7.0 from the initial pH of 5.6. The high percentage loss of activity to the gas phase at low total carbonate concentrations and the marked improvement obtained at increased carrier concentrations and in the presence of NaOH is thus explicable in part in terms of the change in pH of the final counting solution.

The foregoing results established that counting systems containing a minimum of 200 μmol $\text{Na}_2^{14}\text{CO}_3$ and 500 μmol NaOH provide an excellent matrix for the counting of $\text{Na}_2^{14}\text{CO}_3$ solutions. In order to test the ability of NaOH to quantitatively trap relatively large amounts of $^{14}\text{CO}_2$ and to confirm the suitability of the final solutions for assay of activity, $^{14}\text{CO}_2$ was released from quantities of $\text{Na}_2^{14}\text{CO}_3$ as described earlier. Recoveries averaging 98.1%, range 96.8-99.1%, were obtained. These figures derived by the SCR method are based on that found in the 0.05M $\text{Na}_2^{14}\text{CO}_3$ counted in the presence of carrier Na_2CO_3 and NaOH and agree with the stated activity of the material as supplied. Samples were counted on both instruments and the data obtained from both machines using the SCR technique and from the Packard machine using the ESCR technique were identical within experimental error. Results derived using the Isocap machine and the ESCR method were consistently 1.5% lower. Since the same difference exists when ^{14}C hexadecane samples are counted by both techniques in a toluene-only scintillator in the latter machine this finding was not unexpected and the applicability of the ESCR method for the determination of $\text{Na}_2^{14}\text{CO}_3$ activity should be checked on the user's spectrometer.

The system described here represents an advance on that proposed earlier⁴ for the estimation of $^{14}\text{CO}_2$ in that larger quantities of $^{14}\text{CO}_2$ can be handled by a trapping agent which is comparatively inexpensive. The increased levels of $^{14}\text{CO}_2$ capable of being quantitatively trapped suggest that the system will be useful for the assay of $^{14}\text{CO}_2$ produced from the combustion of biological samples or from studies of respiratory metabolism. A further advantage of the present system arises from the fact that samples can be counted after a short dark adaption period (2 h has been routinely adopted) as negligible alkali-induced chemiluminescence has been observed in most batches of the scintillant. The tolerance of this scintillant for NaOH also suggested that the system might be useful for the counting of alkali-digested tissue samples. Preliminary studies indicate that 150 ng samples of fish muscle and slightly smaller quantities of other mammalian tissues can be digested in N NaOH at 60 $^\circ\text{C}$, bleached if necessary with hydrogen peroxide, to give clear counting systems which display minimal chemiluminescence on addition of scintillant. Further aspects of this work are currently being studied.

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DISCUSSION

H.E. HARGRAVES: Barium carbonate- ^{14}C can be counted by γ -scintillation of bremsstrahlung radiation - the method is used at the Radiochemical Centre for BaCO_3 at 60 mCi mmol⁻¹. The pH of the dilution solvent is 9.0-10.0 to contain CO_2 . We

have found there is loss of CO_2 from samples on storage due to reaction of the alkaline solution with the aluminium-lined cap.

J. MURRAY: Thank you for your information. You will of course appreciate that the assay levels can afford to be very much lower, giving much enhanced sensitivity, when the emitted β -particle is counted by conventional liquid scintillation techniques. We have not encountered problems due to a loss of activity in samples monitored for 48 h after preparation. Perhaps the greater amounts of sodium hydroxide and carrier carbonate have helped and it is likely that the xylene-based scintillant may exert a protective action on the cap since we have not noted any marked effect on the foil.