Simple Method for Determination of $^{14}CO_2$ from Expired Air

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Two simple procedures for preparation of homogeneous $^{14}CO_2$ samples for liquid scintillation counting are described. In one procedure (A), the carbon dioxide absorbing agent and the carbonate solution are placed in one tube. Carbon dioxide is released from the carbonate solution by sulfuric acid and absorbed by phenethylamine directly. Added $NaH^{14}CO_3$ (16–8000 dpm/ml.) is recovered nearly quantitatively in the amine phase. Similar results are obtained with another procedure (B), with which $^{14}CO_2$ is released from the carbonate solution and flushed with nitrogen into a counting vial containing absolute methanol and phenethylamine.

COMMON METHODS for measuring expired $^{14}CO_2$ involve trapping by passage of the expired air through sodium hydroxide solution and subsequent reprecipitation as $BaCO_3$. The radioactivity of the preciptated $BaCO_3$ can be determined either on planchets in a gas-flow counter or as suspensions in agents such as thixicin (1), or silica[1] (2) in a liquid scintillation spectrometer. Preparation of planchets or suspensions involves precipitation, filtration, drying and/or powdering, and weighing a portion of the powdered sample, all of which are time-consuming procedures.

Dilute sodium ^{14}C-carbonate solution has also been suspended in silica (3) and radioactivity quantitated in a liquid scintillation spectrometer. The latter procedure is limited to a highly radioactive carbonate solutions and is inadequate to measure low levels of radioactivity in carbonate solutions collected from drug metabolism studies.

Another common method for measuring $^{14}CO_2$ involves acidification of the carbonate solution, diffusion of the generated $^{14}CO_2$ to an organic amine such as hyamine, primine, ethanolamine, ethylene amine, and phenethylamine, and determination of radioactivity with a liquid scintillation spectrometer (4–8).

Phenethylamine is reported to be superior to other amines because it provides (a) high capacity for absorption of CO_2 and (b) minimal quenching (9). Carbon dioxide reacts with phenethylamine to form phenethyl ammonium phenethylcarbamate and with sufficient stoichiometric excess (about 3 moles of amine per mole of CO_2), the reaction is rapid (9).

To insure complete absorption of CO_2 by diffusion following acidification of the carbonate solution, a period of 4–24 hr. is required. The duration depends on (a) the diffusion apparatus and (b) the shaking of the reaction flasks (4–8). Twenty minutes were reported to be adequate if a special diffusion apparatus under reduced pressure (100 mm. Hg) was used. Carbon dioxide was reported to be quantitatively absorbed, but no data were presented (9).

Since all of the above procedures require complicated apparatus, extreme care, and much time, two simple procedures for preparation of homogeneous $^{14}CO_2$ containing samples for liquid scintillation counting have been developed. The details are described in this paper. The procedures require simple apparatus and are practical for the routine simultaneous handling of a large number of samples.

EXPERIMENTAL

Reagents.—All reagents are analytical reagent grade. (a) Alcohol–amine solution; n-amyl alcohol in freshly distilled phenethylamine 1:1 v/v. (b) 9 N sulfuric acid. (c) 5.3 N NaOH solution. (d) Standard $NaH^{14}CO_3$ solution ($^{14}CO_2$ was bubbled into 5.3 N NaOH solution). A series of solutions containing $NaH^{14}CO_3$ was prepared by diluting the $NaH^{14}CO_3$ solution with 5.3 N NaOH solution which contained 5% sodium bicarbonate. (e) Phosphor toluene; 300 mg. [1,4-bis-2(5-phenyloxazolyl)benzene (POPOP)], and 9 Gm. of 2,5-diphenyloxazole (PPO) per liter of toluene.
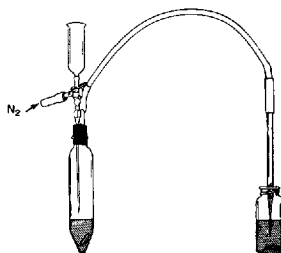
Fig. 1.—Apparatus for generation and trap for $^{14}CO_2$.

TABLE I.—COMPARISON OF THE RECOVERY OF $^{14}CO_2$ BY FLUSHING WITH NITROGEN AND BY ABSORPTION OF PHENETHYLAMINE

NaH^{14}CO$_3$ Added dpm Mean ± S.D.	Expt., No.	Procedure A				Procedure B	
		In Amine Phase		In Aqueous Phase[c]			
		dpm	%	c.p.m./0.2 ml.		dpm	%
*809[a] ± 26	4		100				
*727 ± 17	10						
**457 ± 10	4						
**349 ± 11	4						
Blank	4			31.1 ± 0.5 (S.D.)		32.2 ± 2.6 (S.D.)	
39	2	39	100	31		38	97
77	2	77	100	30		78	101
154	2	157	102	32		166	108
308	2	317	103	33		327	106
616	2	647	105	31		657	107
1232	2	1283	104	33		1308	106
2463	2	2597	105	34		2632	107
4925	2	5154	105	35		5153	105
9850	2	10444	106	36		10613	108
19700[b]	2	20598	105	37			
		Mean ± S.D. 104 ± 2.2		33.2 ± 2.3		105 ± 3.4	

[a] A 100-μl.* or 50-μl.** aliquot of a solution prepared by diluting the stock NaH^{14}CO$_3$ solution with 5.3 N NaOH solution was added directly to a counting vial. Because the inaccuracy of micropipets, the mean dpm (394 ± 48/50 μl.) obtained from four determinations using four different micropipets was used as standard. [b] An amount of 2.5 ml. of the diluted NaH^{14}CO$_3$ solution (see *Footnote a*) was used. [c] The radioactivity remaining in the aqueous phase is insignificant from identical blanks (0.2 < P < 0.1).

TABLE II.—RECOVERY OF $^{14}CO_2$ FROM SODIUM CARBONATE SOLUTION BY PROCEDURE A

NaH^{14}CO$_3$ Added, dpm	Expt., No.	Acid Added		Radioactivity Recovered					
		ml.	Eq. to NaOH, %	Alcohol–Amino Phase			Air Phase[c] Gross c.p.m.	Aqueous Phase	
				vol., ml.	dpm Mean ± S.D.	%		vol., ml.	Gross c.p.m./ 0.25 ml.
238 ± 13[a]	7					100			
Blank	4						32.2 ± 2.6 (S.D.)		
5950[b]	4	1.5	100	11.5	5772 ± 363	97	33	2.5	39
5950	2	1.7	113	11.6	6092	102	32	2.5	38
5950	2	1.9	125	11.8	6052	102	31	2.5	34
5950	2	2.3	150	12.2	6044	102	33	2.3	34
5950	3	3.0	200	13.2	6063 ± 62	102	32	2.1	36
							32.2 ± 0.9(S.D.)		

[a] A 100-μl. aliquot of a solution prepared by diluting the stock NaH^{14}CO$_3$ solution with 5.3 N NaOH solution was added directly to a counting vial. The counting efficient of ^{14}C-toluene in the sample containing 100 μl. of NaH^{14}CO$_3$ solution is 56%. [b] An amount of 2.5 ml. of the diluted NaH^{14}CO$_3$ solution (see *Footnote a*) was used. [c] The air in tube was flushed with nitrogen and trapped in a counting vial containing the alcohol–amine and radioactivity determined. The counts obtained are not significantly different from identical blanks (P < 0.5).

Procedure A.—Exactly 2.5 ml. of the diluted NaH^{14}CO$_3$ solution is placed in a 43-ml. glass-stoppered centrifuge tube. Ten milliliters of the alcohol–amine solution is added to each tube. A 1.1 equivalent of sulfuric acid, 1.7 ml. of 9 N H$_2$SO$_4$, is slowly added *via* a buret to the centrifuge tube. The tube is cooled with ice water during the period of neutralization. Following neutralization, the stoppered tubes are shaken for 10 min. in an International shaker at 280–300 oscillations/min., and centrifuged at 1700 r.p.m. for 10 min. A 10-ml. aliquot of the organic layer, 2 ml. of absolute methanol, and 5 ml. of the phosphor toluene solution are pipeted into a clean counting vial and counted for three 10-min. periods in a Nuclear-Chicago series 720 liquid scintillation spectrometer. Samples are counted for three 10-min. periods to provide a 0.95 counting error of less than 2% for gross counts (10). Radioactivity in a given sample is considered significant when a value of 6 net c.p.m. or greater is obtained. The remaining alcohol–amine and aqueous solutions are drawn (with the aid of a propipet) into a 5-ml. graduated pipet. The volume of the alcohol–amine phase is estimated in the pipet after the organic and aqueous layers have separated.

Procedure B.—Exactly 2.5 ml. of the diluted NaH^{14}CO$_3$ solution is placed in a 43-ml. glass-stoppered centrifuge tube. Ten milliliters of absolute methanol in phenethylamine (1:1 v/v) solution is placed in a counting vial. The tube is stoppered with a rubber stopper which has previously been punctured with a No. 17 gauge needle and connected with a small Chieftain intravenous plastic tubing (^{1}/$_8$ × ^{1}/$_{32}$ in.).[2] The other end of the tubing is connected to a 6-in. glass tip,[3] in which a plug of glass wool is inserted. The tip of the Dispo-pette is immersed in the methanol–amine solution. The arrangement of the apparatus is shown in Fig. 1. Two milliliters of 9 N sulfuric acid is slowly added to the tube *via* a 5-ml. syringe while the solution is stirred with a magnetic stirrer. Following acidification, the generated CO$_2$ and the air in the tube is flushed 10 min. with about 0.5 lb./sq. in. pressure of nitrogen. The Dispo-pette and the magnetic stirring bar are rinsed with 1 ml. of absolute methanol each, and 5 ml. of the phosphor toluene is then added. The mixture is counted

[2] Chieftain intravenous tubing, American Hospital Supply Corp., Evanston, Ill.
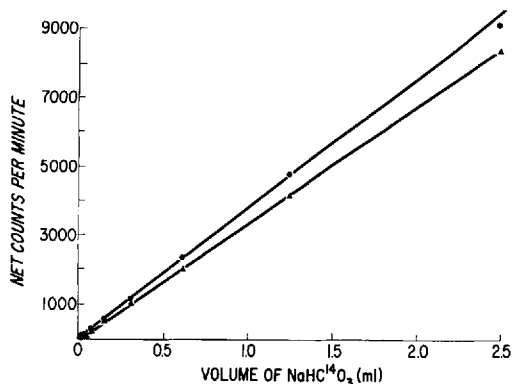[3] Dispo-pettes, Scientific Products, Evanston, Ill.

Fig. 2.—Plot of net c.p.m. *versus* volume of standard $NaH^{14}CO_3$ solution added. Key: ●, flushing with nitrogen; ▲, absorption by phenethylamine.

as described under *Procedure A*. The specific radioactivity of the standard $NaH^{14}CO_3$ is determined by first mixing a 100-μl. or a 50-μl. aliquot of the solution with two milliliters of absolute methanol, and then with 10 ml. of the alcohol–amine solution, and 5 ml. of the phosphor toluene.

All samples are corrected for quenching by addition of ^{14}C-toluene as an internal standard. Paired *t* tests were used to compare values obtained from samples and those of identical blanks.

RESULTS

The percentage recovery of $^{14}CO_2$ from sodium hydroxide–carbonate solution by procedure *A* is 104 ± 2.2 (S.D.) as shown in Table I. No significant radioactivity is found in the aqueous layer and in the air of the tube (Table II). A linear relationship is found between the mean of the net counts per minute (c.p.m.) obtained and the volume of $NaH^{14}CO_3$ solution added to the sample as shown in Fig. 2. The counting efficiency of ^{14}C-toluene in the sample containing 10 ml. of the alcohol–amine, 2 ml. of absolute methanol, and 5 ml. of phosphor toluene is approximately 60% of the ^{14}C-toluene in phosphor toluene alone.

The percentage recovery of $^{14}CO_2$ from sodium hydroxide–carbonate solution by procedure *B* is 105 ± 3.5 (S.D.) as shown in Table I. A linear relationship is found between the mean of the net c.p.m. obtained and the volume of $NaH^{14}CO_3$ in the sample as illustrated in Fig. 2. The counting efficiency of ^{14}C-toluene in the sample containing 10 ml. of the "trapping" methanol–amine solution, 2 ml. of absolute methanol, and 5 ml. of phosphor toluene is approximately 60% of the ^{14}C-toluene in phosphor toluene alone.

DISCUSSION

Procedure A.—The theory used in procedure *A* is based on the fact that dissociation of strong acids and strong bases is greater than that of weak bases. If sulfuric acid is mixed with a strong base such as sodium hydroxide–carbonate solution and a weak base such as phenethylamine, the sulfate ions will replace the hydroxide and carbonate ions in preference to reacting with the amine. Preliminary studies gave identical results whether sulfuric acid was added above or below the amine phase.

The result suggests that the affinity of sulfate ions for sodium ions is stronger than both the affinity of sulfate ions for amine ions and carbonate ions for sodium ions.

The higher recovery of $^{14}CO_2$ from known samples was presumably due to the inaccuracy of the micropipet used. The data indicate that $^{14}CO_2$ is recovered quantitatively from $NaH^{14}CO_3$ solutions by both procedures *A* and *B*.

Varying amounts of sulfuric acid (from 113 to 200% necessary to neutralize the NaOH solution) in studies on the recovery of $^{14}CO_2$ from sodium hydroxide–carbonate solution give identical results (Table II). The low recovery of $^{14}CO_2$ while the acid is just 100% equivalent (calculated) to the NaOH solution might have resulted because the actual strength of the acid was not sufficient (the acid and alkali solutions were not standardized by titration). Both the acid and alkali solutions were standardized for routine use. As progressively greater volumes of acid were used, there occurred an increase both in the volume (from 11.5 to 13.2 ml.) and the quenching of the amine–CO_2 phase.

The above procedure was also studied as to (*a*) the possible loss of $^{14}CO_2$ to air *via* diffusion through the alcohol–amine layer using the techniques of procedure *B*, and (*b*) the retention of radioactivity in the aqueous phase. The data shown in Table II indicate that there is no $^{14}CO_2$ loss to air [the air in the tube given 31–33 gross c.p.m. which is not significantly different from the counts obtained on identical blanks (32 gross c.p.m.) $(P < 0.5)$]. The radioactivity in the aqueous phase, determined by counting an 0.25-ml. aliquot with 10 ml. of the methanol–amine, 2 ml. of absolute methanol, and 5 ml. of phosphor toluene, is not significantly different from the identical blanks (Tables I and II, $0.2 < P < 0.1$).

Procedure B.—If one prefers to avoid shaking, centrifuging of the mixture, and transferring of the alcohol–amine–CO_2 to a counting vial in procedure *A*, procedure *B* is suitable. Varying the flushing time from 10 to 30 min. results in identical recovery of added $^{14}CO_2$ (Table III). It is believed that a shorter flushing period than 10 min. may be sufficient if a smaller flask is used.

TABLE III.—EFFECT OF FLUSHING TIME WITH NITROGEN ON THE RECOVERY OF $^{14}CO_2$ GENERATED FROM CARBONATE SOLUTION

$NaH^{14}CO_3$ dpm Added Mean $\pm$ S.D.	Expt., No.	Flushing Time, min.	Recovery dpm Mean $\pm$ S.D.	%
175.1 ± 6.7^a	3			100
4378^b	4	2	4085 ± 115	93
4378	4	5	4312 ± 224	99
4378	4	10	4394 ± 229	100
4378	4	20	4522 ± 385	103
4378	3	30	4486 ± 56	103

^{a} A 100-μl. aliquot of a solution prepared by diluting the stock $NaH^{14}CO_3$ solution with 5.3 *N* NaOH solution was added directly to a counting vial. The counting efficient of ^{14}C-toluene in the sample containing 100 μl. $NaH^{14}CO_3$ solution is 56%. ^{b} An amount of 2.5 ml. of the diluted $NaH^{14}CO_3$ (see *Footnote a*) solution was used.

SUMMARY

Two procedures have been used in this laboratory for measurement of the expired $^{14}CO_2$ of rats and mice, in various drug metabolism studies and have been found to give rapid, consistent results.

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Some Physicochemical Properties
of the Montmorillonites

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The swelling, moisture sorption, particle size, cation-exchange, and drug adsorption properties of selected montmorillonites were studied. The swelling of montmorillonite was found to depend on both adsorptive and osmotic phenomena. The study of the sorption of alkaloidal drugs by the clay showed that brucine was sorbed by both adsorption and ion-exchange reactions, forming a monomolecular layer on the interior surface of the clay; methapyrilene and triethylamine were sorbed by an ion-exchange reaction; and niacinamide was neither adsorbed nor ion-exchanged.

THE HYDROUS magnesium aluminum silicate minerals, montmorillonites, because of their unusual properties and widespread occurrence have attracted the attention of workers in many fields of application. These clays are extremely plastic when moist, swell in the presence of water, and can be dispersed in water forming thixotropic gels. Bentonite U.S.P. is a mineral composed of 90% montmorillonite (1).

The applications of the montmorillonites in the pharmaceutical field have been explored extensively. Magnesium aluminum silicate[1] and bentonite, alone or in combinations with other common suspending agents, have been studied for their use as suspending agents (2–5). Guth *et al.* prepared different types of bentonites by saturating the clay with Na^+, K^+, Ca^{2+}, Mg^{2+}, and H^+. They used these various cation saturated bentonites to conduct a series of studies on the buffer capacity, cationic-exchange properties, and drug binding and release properties of bentonite (6–10). Many studies of montmorillonites as tablet disintegrants have been made, with the

work of Feinstein and Bartilucci (11) being the most recent.

Although the montmorillonite class of clay minerals is widely used in the field of pharmacy, little has been reported in the pharmaceutical literature concerning their basic physical and chemical properties and the relation between such properties and pharmaceutical applications and utility. It was, therefore, the purpose of this work to conduct a pharmaceutically oriented study of the physicochemical properties of the montmorillonites, so that the various present applications and limitations or restrictions of the clays might be better understood and future applications more accurately determined.

EXPERIMENTAL

The three commercial montmorillonites studied in this work were magnesium aluminum silicate, magnesium aluminum silicate F, and WG.[2] These three types of clay will be referred to as clay I, II, and III, respectively, in the remainder of this paper.

Magnesium aluminum silicate has a composition of 61.1% silicone dioxide, 13.7% magnesium oxide, 9.3% aluminum oxide, 2.9% sodium oxide, 2.7% calcium oxide, and smaller amounts of titanium, ferric, and potassium oxides, 1.8% carbon dioxide, and 7.2% water of combination (12). The moisture content of the material will increase if exposed to moist atmospheres. The various grades of