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REVIEW

Stability and storage of compounds labelled with radioisotopes

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SUMMARY

This review summarises the present knowledge concerning the decomposition by self-irradiation of compounds labelled with the radioisotopes, carbon-14, hydrogen-3 (tritium), sulphur-35, selenium-75, chlorine-36, phosphorus-32, iodine-125, iodine-131, cobalt-57 and cobalt-58. A discussion of the methods which can be used for the control of this decomposition is also included.

INTRODUCTION

Most users of compounds labelled with radioisotopes recognise that such compounds decompose on storage and that the decomposition is accelerated by self-irradiation. The degree of the decomposition in relation to the storage conditions of the compound, and the measures which can be taken to control and minimise the rate of self-radiolysis, are perhaps not always so well known. This review summarises present knowledge of the decomposition of labelled compounds and methods of reducing it.

Information on this subject is largely empirical. The increasing sensitivity of methods for the analysis and measurement of radioactive chemicals is making more users aware of the problem and its importance is being increasingly recognised. Not only are a large number of labelled compounds, particularly organic compounds, extensively used as tracers, but many applications demand a very high purity. Fractions of a *percent* radiochemical impurity can sometimes lead to wrong deductions being made from a tracer investigation, and under these conditions the problem of decomposition by self-irradiation becomes a very serious one. An example is the need for tyrosine, labelled with tritium or carbon-14, at a very high radiochemical purity for the estimation of tyrosine hydroxylase. The method measures the formation of 3,4-dihydroxyphenylalanine, which is itself a product of the self-irradiation of the tyrosine in aqueous solution [1]. Another example is the need for glucose-1-C 14 of high radiochemical purity when it is used for an insulin bio-assay. SONKSEN[2] has shown that fractions of

a *percent* of impurity have a marked effect on the 'no tissue blank' and that this can be avoided if a purified labelled sugar is used.

1. Radionuclides

Compounds labelled with the pure beta emitting radioisotopes, carbon-14, tritium, sulphur-35, phosphorus-32 and chlorine-36, are most commonly used in tracer investigations. Compounds labelled with the gamma emitting radioisotopes such as iodine-125, iodine-131, cobalt-57, cobalt-58 and selenium-75 have special application in medicine. Some properties of these radionuclides are shown in Table 1.

Table 1. *Physical Properties of Some Radionuclides*

Radionuclide	Half-life	Beta Energy MeV		Specific Activity		Daughter nuclide (stable)
		Max	Mean	Max mc/mA	Common values for compounds mc/mM	
Tritium	12.26 years	0.018	0.0057	2.9×10^4	$10^2 - 10^4$	Helium-3
Carbon-14	5700 years	0.159	0.050	64	$1 - 10^2$	Nitrogen-14
Sulphur-35	87.2 days	0.167	0.049	1.5×10^6	$1 - 10^2$	Chlorine-35
Chlorine-36	3.03×10^5 years	0.714	0.3	1.2	$10^{-3} - 10^{-1}$	Argon-36
Phosphorus-32	14.3 days	1.71	0.69	9.3×10^6	$10 - 10^2$	Sulphur-32
Iodine-131	8.04 days	0.81	0.19	1.7×10^7	$10^2 - 10^4$	Xenon-131
Iodine-125	60 days	Electron capture		2.2×10^6	$10^2 - 10^4$	Tellurium-125
Cobalt-57	270 days	Electron capture		4.9×10^5	$10^3 - 10^5$	Iron-57
Cobalt-58	71 days	Electron capture $+ \beta^+$		1.9×10^6	$10^3 - 10^5$	Iron-58
Selenium-75	121 days	Electron capture		1.1×10^6	$10 - 10^3$	Arsenic-75

Decomposition depends in part on the amount of energy absorbed by the compound during its useful life, so that for a given amount of activity the radiation energy emitted should be a guide to the seriousness of the problem. The problem of self-irradiation decomposition might be expected to increase in magnitude as the series of pure beta emitters in the Table 1 is descended, but in fact almost the reverse is true. This is largely for three reasons:

(i) the fraction of energy absorbed is much less than unity for the more energet-

ic beta emitters such as phosphorus-32 ; on the other hand almost complete total absorption of the beta energy occurs with tritium compounds. Gamma energy is, in general, little absorbed by the compound itself or its immediate environs.

(ii) the decomposition also depends on the specific activity of the compound. As can be seen from the Table 1, the specific activities of tritiated compounds in current use are usually much higher than those for compounds labelled with other pure beta emitting radionuclides.

(iii) the absorbed energy decreases exponentially with time. This is an important factor for compounds labelled with radionuclides having a short half-life such as iodine-131 or phosphorus-32.

2. How and why do radioisotopically labelled compounds decompose?

The reason why labelled compounds decompose is not difficult to understand : the radiation energy will be commonly absorbed by the compound itself or by its environs. If the former occurs, then the excited molecules may break-up in some manner ; if the latter occurs the radiation energy can produce free radicals and other reactive species which may then cause destruction of the molecules of the labelled compound.

The observations of Calvin and his colleagues[3] in 1953 showed that extensive self-radiolysis can occur of compounds labelled with the long lived carbon-14, from which beta particles of quite modest mean energy are emitted. This shattered any illusions that radioisotopically labelled compounds were as stable as their unlabelled counterparts. However, surprisingly few publications have subsequently appeared dealing specifically with this important subject. On the other hand much has been published concerning the effects of external radiation, such as gamma or X-irradiation for example, on compounds of all types [4–8]. Unfortunately the information from such experiments provides only a rough approximation as to the expected decomposition of labelled compounds by self-irradiation.

The modes by which the decomposition of labelled compounds can arise were classified by Bayly and Weigel[9] in 1960 ; this classification is summarised in Table 2.

Primary (internal) decomposition is the production of an impurity due to the disintegration of the unstable nucleus. A decomposition fragment so produced will be radioactive only if the molecule which is decomposing contains two or more radioactive atoms. For many investigations compounds are used which contain quite a small proportion of doubly labelled molecules and for such compounds the radioactive impurities from primary (internal) decomposition can usually be neglected.

All the effects of radiation decomposition are dependent upon specific activity and in order to make comparisons between compounds it is the molar specific activity which should be considered — that is mc/mM rather than

TABLE 2. *Modes of Decomposition of Labelled Compounds*

Mode of Decomposition	Cause	Method for Control
Primary (internal)	Natural isotopic decay.	None, for a given specific activity. *
Primary (external)	Direct interaction of the radioactive emission (alpha, beta or gamma) with molecules of the compound.	Dispersal of the labelled molecules.
Secondary	Interaction of excited products with molecules of the compound.	Dispersal of active molecules; cooling to low temperatures; free radical scavenging.
Chemical	Thermodynamic instability of compounds and poor choice of environment.	Cooling to low temperatures; removal of harmful agents.

* Note that dilution with the inactive form of the compound subsequent to preparation is not beneficial in this case [9].

mc/mg. This is particularly important for macromolecules, labelled for example with carbon-14, where a modest isotopic abundance may be associated with quite a high molar specific activity.

Secondary decomposition is commonly the most damaging and the most difficult to control. It is also the mode most susceptible to minor variations of the environmental conditions.

In considering all the possible radiation effects causing decomposition, ordinary chemical decomposition of the compound is often overlooked. SMITH[10] gives a brief but very illuminating account of factors (such as oxidation, hydrolysis, biological reactions, etc.) which influence the chemical decomposition of medical preparations. Chemical decomposition arising from such factors, is even more likely to occur with radioactive chemicals because these are often used in solution at very low chemical concentration, or are prepared in such small chemical amounts that it is difficult to ensure complete freedom from inactive impurities which might be harmful. Even hard glass surfaces have been found to adversely affect the stability of radioactive carbohydrates although the non-neutrality of the glass could only be demonstrated by extraction with boiling water [9]. It is also essential to guard against photochemical or microbiological decomposition of the compound.

3. PERCENTAGE DECOMPOSITION IN RELATION TO THE G(-M) VALUE

In radiation chemistry it is usual to express decomposition in terms of 'G' values — the yield in number of molecules (atoms, ions, etc.) per 100 ev

absorbed by the system. In investigations of self-radiolysis it is sometimes useful for comparisons between compounds to calculate G(-M) values, the '-M' representing molecules of the starting compound irreversible altered by the radiation process. The 'system' may be the labelled compound itself, when it is stored in the pure state, or a solution of it in water or other solvents. G(-M) values are very dependent on the storage conditions for the compound.

Given a G(-M) value for a particular compound under specified conditions, it is possible to calculate the magnitude of the self-decomposition from the following equation (1) :

$$P_d = f.\bar{E}.S_a.5.3 \times 10^{-9}.G(-M) \qquad (1)$$

where P_d is the initial percentage decomposition per day

f is the fraction of the radiation energy absorbed by the system

$\bar{E}$ is the mean energy of the emission in electron volts

S_a is the initial specific activity of the compound in millicuries per millimole

Inserting the appropriate $\bar{E}$ values, the equation (1) reduces to :

$P_d = 3.0 \times 10^{-5}.G(-M).S_a$ (2) for tritium

$P_d = 2.65 \times 10^{-4}.G(-M).S_a.f$ (3) for carbon-14

$P_d = 2.6 \times 10^{-4}.G(-M).S_a.f$ (4) for sulphur-35

To calculate the degree of decomposition for a particular time the equation (1) can be used in its simple linear form, providing the magnitude of decomposition is modest (say $< 10\%$) and the storage time is short compared to the half-life of the isotope. If these restrictions are not met the exponential forms need to be used [9]. For example, for sulphur-35 the « equivalent storage time » (i.e. the time at which it can be regarded as being stored at its initial specific activity) is given by $126\ (1 - e^{-t/126})$ days, where t is the actual time of storage in days. A similar adjustement must be made for the extent of decomposition when it exceeds about 10%.

For tritium compounds the value of 'f' can be taken as unity because of the low penetrating power of the weak beta radiation, but for compounds labelled with other radioisotopes and stored under favourable conditions, the value of 'f' may be considerably less than unity. Unfortunately it is difficult to calculate or obtain a reasonable estimate for the value of 'f' except in the simplest of cases [9]. Consequently it has been the usual practice to assume a value of unity for 'f' for calculation purposes ; however, for practical purposes this is far from the truth and the possible implications of this should not be forgotten.

It cannot be stressed too strongly that minor variations in the storage conditions of a compound can exert a major effect on the rate of decomposition and on the G(-M) value, and the tabulated results in this review should be treated only as a rough guide. It should also be remembered that determination of the percentage decomposition (and hence the G(-M) value) is related to the sensitivity of the methods which are employed for the detection and measure-

ment of the radioactive impurities. Such methods have undoubtedly been improved in their sensitivity over the past few years and this should be borne in mind when considering the significance of a few percent decomposition of very old samples which have been reanalysed — using, perhaps, a more sensitive method for detecting radioactive impurities than was used originally.

ROCHLIN[11] summarises the methods which have been used for the calculation of absorbed dose, percentage decomposition and G(-M) value.

4. ANALYSIS OF LABELLED COMPOUNDS

Before describing some of the results of decomposition studies, it is useful to briefly consider the methods used for measuring the amounts of radiochemical impurities.

Some of the pitfalls in the analysis of carbon-14 compounds are discussed in detail by CATCH[12], and those for tritium compounds by EVANS[13], but the following points are of general interest :

(i) In general, physical methods of analysis such as melting point, boiling point, refractive index and ultraviolet or infra-red spectrophotometry are inadequate and are not sufficiently sensitive to measure radiation decomposition.

(ii) Reverse isotope dilution analysis is a highly desirable method for the determination of the purity of a labelled compound. For detecting decomposition however, it is less useful unless one is concerned only with a few specific impurities.

(iii) Chromatographic or electrophoretic methods for detecting decomposition have generally been preferred. However, neither of these methods are without pitfalls. For example, paper chromatography must not result in decomposition of the product on the paper such as that observed with carbon-14 labelled ribulose and ribulose diphosphate [14]. Gas liquid chromatographic methods must also be geared for detecting radioactivity [15] and must be quantitative for accurate interpretation.

(iv) The visual appearance of a labelled compound can sometimes give a misleading indication of radiation decomposition. Whilst the presence of colour (for example) in a normally colourless compound may indicate some impurity, it can often represent quite a negligible amount and this may not be radioactive. Some examples are the red-violet (iodine) colouration of tritiated or carbon-14 labelled methyl iodide [16] (and other alkyl or aryl iodides [17]), and the straw colour of labelled benzene or acetic anhydride [17]. It is possible for a solid labelled compound to be deeply coloured, due to deformations in the crystal lattice, without signifying the presence of any impurity. The dark green colour produced on storage of solid thiosemicarbazide-S35 is an example of this phenomenon.

One cannot rely upon one method of analysis only : for example, a paper chromatographic method used for determining the radiochemical purity of an

L-amino acid will not indicate whether racemization has occurred — reverse dilution analysis is also required. Volatile impurities cannot be detected quantitatively by paper or thin-layer chromatographic methods. With tritium

TABLE 3. *Self-Radiolysis of Organic Compounds Labelled with Radioisotopes*

Compound Class	Radioisotopes	References	Table No.
Aliphatic acids, esters and salts	Carbon-14	3, 18	6
Aliphatic acids, esters and salts	Tritium	13, 19-22	10
Aliphatic alcohols	Carbon-14	23	6
Aliphatic alcohols	Tritium	13, 19, 20, 23	10
Aliphatic hydrocarbons	Carbon-14	—	6
Aliphatic hydrocarbons	Tritium	13, 20, 24	—
Alkyl halides	Carbon-14	16	6
Alkyl halides	Tritium	13, 20	—
Aromatic compounds	Carbon-14	3	6
Aromatic compounds	Tritium	13, 20, 25-27	10
Amino acids	Carbon-14	3, 28	6,7
Amino acids	Tritium	13, 19, 20, 26, 29-31	10
Amino acids	Sulphur-35	32	4
Amino acids	Iodine-125, 131	71	11
Carbohydrates	Carbon-14	9, 33-35	8
Carbohydrates	Tritium	13, 20	—
Heterocyclic compounds	Carbon-14	3	6
Heterocyclic compounds	Tritium	13, 20, 36	10
Heterocyclic compounds	Sulphur-35	—	4
Heterocyclic compounds	Phosphorus-32	—	5
Heterocyclic compounds	Cobalt-57, 58	37, 38	12, 13
Macromolecules	Carbon-14	9	—
Macromolecules	Tritium	39	—
Nucleosides	Carbon-14	40	9
Nucleosides	Tritium	13, 20, 26, 40-46	10
Nucleosides	Iodine-125, 131	47	11
Nucleotides	Phosphorus-32	—	5
Nucleotides	Tritium	—	10
Proteins and Peptides	Tritium	39, 48	—
Steroids	Carbon-14	3, 49, 73	6
Steroids	Tritium	13, 20, 50-52, 72, 74	10

compounds it is also necessary to check for 'labile' tritium particularly when the compounds are stored in solution. Such 'labile' tritium may be formed by slow exchange of the tritium atoms of the compound itself or its decomposition products, with the hydrogen atoms of the solvent.

5. Experimental observations

For easy reference, studies of decomposition by self-irradiation on certain classes of organic compounds labelled with various radioisotopes, are collected in Table 3.

(a) *Chlorine-36 Compounds*

There does not appear to be any published information concerning the decomposition by self-irradiation of compounds labelled with chlorine-36. This is perhaps not surprising in view of the very long half-life of this radionuclide and the very low specific activity of the compounds labelled with it (see Table 1).

(b) *Sulphur-35 Compounds*

Little information has been published concerning the self-radiolysis of compounds labelled with sulphur-35, in fact only methionine-S35 has been previously studied in any detail [32]. Some results obtained by Dr. J.R. Ogle[17] are given in the Table 4, and it is seen that only a few of the wide variety of compounds studied show any marked decomposition in spite of the fact that many of the compounds were stored in bulk at high specific activity.

Sodium salts of long chain aliphatic sulphates show a marked sensitivity to self-radiolysis which is similar to that observed for the decomposition of some long chain aliphatic carboxylic acids labelled with carbon-14 (see Table 6).

The stability of thiosemicarbazide-S35, a compound extensively used in the analysis of keto steroids [53], has been examined under several storage conditions. However, there is little to choose between storage in aqueous solution at $-30°C$, as a solid at $-30°C$, or at room temperature *in vacuo* over phosphorus pentoxide; but storage in methanol even at $-30°C$ results in a more rapid decomposition.

DL-Methionine-S35 at 100 mc/mM is unstable at room temperature in the presence of moisture but the anhydrous material is quite stable as a crystalline solid stored *in vacuo*. However, L-methionine-S35 undergoes up to 10% decomposition per month when stored as the anhydrous solid amino acid at a comparable specific activity; the decomposition rate is somewhat reduced at $-30°C$ (see Table 4). L-Methionine-S35 therefore appears to be more sensitive to self-radiolysis than DL-methionine-S35, in the solid state. Their

TABLE 4. *Self-Decomposition of Compounds Labelled with Sulphur-35*

Compound	Specific activity mc/mM	Storage state	Temp. °C	Storage time days	Decomp. %	G(-M) *
« AET » (2-Aminoethyl-*iso*thiouronium bromide hydrobromide)	4	Solid *in vacuo* over P_2O_5	20	354	N.D.	—
Chlorpromazine	7	Solid *in vacuo* over	20	265	N.D.	—
Chlorpromazine	10	P_2O_5	20	105	N.D.	—
Cysteamine	2	Solid *in vacuo* over P_2O_5	20	321	N.D.	—
Cysteamine	25	Solid *in vacuo* over	20	245	N.D.	—
dihydrochloride	29	P_2O_5	20	164	N.D.	—
Cysteamine	30	Solid *in vacuo* over	20	341	N.D.	—
dihydrochloride	50	P_2O_5	20	203	N.D.	—
L-Cystine	184	Solid *in vacuo* over P_2O_5	20	157	N.D.	—
S-Ethyl-L-cystine	1.5	Solid *in vacuo* over P_2O_5	20	149	N.D.	—
L-Homocystine	7	Solid *in vacuo* over P_2O_5	20	148	N.D.	—
6-Mercaptopurine	7	Solid *in vacuo* over P_2O_5	20	126	N.D.	—
L-Methionine	96	Solid *in vacuo* over	−30	33	5	7.0
L-Methionine	96	P_2O_5	−30	77	10	7.3
L-Methionine	96	Water (2.03 mc/ml)	−30	77	N.D.	—
L-Methionine	135	Water (4.6 mc/ml)	−30	152	2	0.7
L-Methionine	207	Water (7.6 mc/ml)	−30	75	2	0.7
L-Methionine	46	Water ° (1.2 mc/ml)	20	84	6	8.3
L-Methionine	58	Water ° (3.0 mc/ml)	20	126	7	6.0
Potassium ethyl xanthate	18	Solid *in vacuo* over P_2O_5	20	190	N.D.	—
Sodium dodecylbenzene-sulphonate	8	Water (3.3 mc/ml)	20	175	12	65
Sodium ethane sulphonate	2-25	Solid *in vacuo* over P_2O_5	20	128	N.D.	—
Sodium hexadecylsulphate	56	Solid *in vacuo* over P_2O_5	20	120	11	10.3
Sodium lauryl sulphate	6	Solid *in vacuo* over	20	126	N.D.	—
	26	P_2O_5	20	183	10	16.1
	45	Solid *in vacuo* over P_2O_5	20	78	7	10.5
Sodium octyl sulphate	10	Solid *in vacuo* over P_2O_5	20	179	3 (sulphate)	—

TABLE 4. (*continued*)

Compound	Specific activity mc/mM	Storage state	Temp. °C	Storage time days	Decomp. %	$G(-M)$ *
Sodium sulphite	11	Freeze-dried solid under N_2	20	29	26	406
Sodium sulphite	11	Water (5.5 mc/ml) under N_2	20	29	86	2650
Sodium thiosulphate (labelled at 'inner' or 'outer' S atom)	27	Solid *in vacuo* over P_2O_5	20	63	N.D.	—
	37.5		20	91	N.D.	—
	37.5		20	129	N.D.	—
	37		20	202	N.D.	—
Sulphanilic acid	160	Solid *in vacuo* over P_2O_5	20	50	<2%**	<1.2
	140		20	268	<2%**	<0.5
Taurine	30	Solid *in vacuo* over P_2O_5	20	153	N.D.	—
Tetramethylthiuram disulphide	17	Solid *in vacuo* over P_2O_5	20	209	N.D.	—
Thiamine (Vitamin-B_1)	25	Solid *in vacuo* over P_2O_5	20	112	N.D.	—
Thioacetamide	2	Solid *in vacuo* over P_2O_5	20	153	N.D.	—
Thiopentone ('Pentothal, sodium')	12	Solid *in vacuo* over P_2O_5	20	65	N.D.	—
Thiosemicarbazide	160	Solid *in vacuo* over P_2O_5	20	54	5**	2.8
Thiosemicarbazide	155		20	20	2 **	2.7
Thiosemicarbazide	155		20	74	15 **	7.2
Thiosemicarbazide	220		20	35	4 **	2.3
Thiosemicarbazide	191		20	211	7 **	1.4
Thiosemicarbazide	196		−30	18	4	4.7
Thiosemicarbazide	196		−30	63	6	2.4
Thiosemicarbazide	196		−30	112	8	2.2
Thiosemicarbazide	196	Water (2 mc/ml)	−30	18	5	6.0
Thiosemicarbazide	196	Water (2 mc/ml)	−30	63	7	2.8
Thiosemicarbazide	196	Water (2 mc/ml)	−30	112	8	2.2
Thiosemicarbazide	196	MeOH (2.2 mc/ml)	−30	18	15	19.0
Thiosemicarbazide	196	MeOH (2.2 mc/ml)	−30	63	20	8.5
Thiosemicarbazide	196	MeOH (2.2 mc/ml)	−30	112	30	9.4
Thiourea	20	Solid *in vacuo* over P_2O_5	20	134	N.D.***	—
Thiourea	35		20	101	N.D.***	—
Thiourea	46		20	134	N.D.***	—

N.D. = No detectable decomposition
° = Sterilised by autoclaving at 120 °C (15 lbs psi) for 30 minutes
* = Calculated from equation [4] assuming '*f*' is unity
** = Accompanied by considerable discolouration
*** = Slight discolouration of samples

different behaviour is probably due to the difference in the crystalline form of the two compounds [54], perhaps similar to the radiation sensitivity of the two forms of choline chloride [55]. There is no significant difference between the rate of decomposition of DL- and L-methionine-S35 when stored in aqueous solution; storage at low temperature (−30°C) is best for both. At −30°C in aqueous solution less than 0.02% racemization of the L-isomer is observed during 152 days [17].

Although the yields of radiolysis products are different, there is a similarity between the compounds produced by self-radiolysis of labelled methionine and those produced by gamma or X-irradiation [32, 56–58]. Products arise mainly through demethylation, deamination and oxidation to the sulphone.

Sodium sulphite-S35 is very sensitive to decomposition — particularly when dissolved in water; unfortunately the decomposition rates reported in Table 4 were not checked for chemical decomposition against unlabelled controls.

Radiation damage to barium sulphate-S35 producing a highly 'active' surface is offered as an explanation for the observed increased solubility of barium sulphate-S35 compared with the unlabelled sulphate [59]. Similar effects were not observed with lead sulphate-S35.

(c) *Phosphorus-32 Compounds*

Phosphorus-32 compounds are normally used within four weeks of their preparation because of the short half-life (14.3 days) of the radionuclide. The high energy of the beta particles results in only a small fraction of the radiation energy being self-absorbed by the compound. Therefore, in general, compounds labelled with phosphorus-32 are best stored in their natural form as thin films. The use of solvents for these compounds has not, in general, proved beneficial.

Some results obtained by R. MONKS[17] are recorded in Table 5. The presence of oxygen and chemical impurities are observed to increase the rate of decomposition.

Di*iso*propylphosphorofluoridate ('DFP')-P32 is one of the more sensitive compounds and dilution to the material in propylene glycol or arachis oil has little effect on the stability of the compound. The rate of decomposition is about 10% per week for solutions with an initial radioactive concentration of 300 μc/ml; this corresponds to an internal dose of approximately 6×10^4 rads in the first week. Solutions of 'DFP' in propylene glycol can be sterilised by gamma irradiation (2.5 Megarads) with less than 5% additional decomposition.

Sodium pyrophosphate-P32 in aqueous solution at pH 10 can be heated for 1 hour at 100°C in a sealed tube without detectable decomposition. At pH 10 solutions remain stable over a period of about 4 weeks [60] but at pH values below 8.5 decomposition rates increase progressively as solutions are made more acidic. It is not certain whether this decomposition in acidic solutions is accelerated by self-radiolysis.

TABLE 5. *Self-Decomposition of Compounds Labelled with Phosphorus-32*

Compound	Specific activity mc/mM	Storage condition	Temp. °C	Storage time weeks	Observed decomposition %
Adenosine triphosphate-γ-P32 (tetraammonium salt)	800	Water (0.3% soln.)	−30	4	less than 2
Aze-TEPA-P32 (P,P-bis(1-aziridinyl)-N-ethyl, N-1,3,4-thia-(diazol-2-yl phosphinic amide)	4.5	Sealed tube under air	20	7	11
0-Butylethylphosphonothiolothionate (ammonium salt)	2	Sealed tube under air	20	6	N.D.
2-Cyanoethylphosphate (barium salt)	600	Sealed tube under air	20	4	less than 1
'Diazoxon'	5	Sealed tube under air	20	4	3 *
Di*iso*propylphosphorofluoridate (DFP)	50-100	Sealed tube under air	20	1	10-20
Di*iso*propylphosphorofluoridate (DFP)	50-100	Propylene glycol *in vacuo*	20	1	10
'Dipterex' (0,0-Dimethyl-2,2,2-trichloro-1-hydroxyethyl-phosphonate)	2.5	Sealed tube under air	20	4	2
	2.5		20	10	6
Diethylstilboestrol diphosphate disodium salt	80	Sealed tube under air	20	2	less than 5
'Disyston' (0,0-Diethyl-2-ethylthioethyl phosphorodithioate)	11	Sealed tube under air	20	8	less than 1
Endoxan (N,N-bis(2-chloroethyl-N,0-trimethylene phosphorodiamidate)	1.5	Sealed tube under air	20	7	4
Thymidine-5'-monophosphate (ammonium salt)	992	Freeze-dried solid	0	7.5	8
Thymidine-5'-monophosphate (ammonium salt)	992	Water (3.4 mc/ml)	20	1.5	2
	992	Water (3.4 mc/ml)	20	7.5	25
	992	Water (3.4 mc/ml)	0	7.5	20
Thio-TEPA (Triethylene thiophosphoramide)	4	Sealed tube under air	20	7	3
Trimethyl phosphite	5	*In vacuo*	20	8	N.D.
Trimethyl phosphite	10	In air	20	2	30
Sodium hypophosphite	100	Sealed tube under air	20	2	less than 2

N.D. = No detectable decomposition
* = Decomposition rate sensitive to chemical impurities, particularly diethylphosphorochloridate.

(d) *Carbon-14 Compounds*

Decomposition of compounds labelled with carbon-14 is not in general an insuperable problem provided suitable conditions are used for their storage.

ROCHLIN[11] has adequately reviewed the published information up to April 1965 concerning the decomposition of carbon-14 compounds, which includes a detailed summary of the early work of TOLBERT *et al* [3]. It would be superfluous to re-summarise this information for the present review and instead some results obtained at the Radiochemical Centre for a wide variety of compounds are listed in Table 6.

The storage of selected carbon-14 compounds in aqueous solution may not be so unsatisfactory as was once thought [12]. Deep frozen (−40 °C) solutions of thymidine-2-C14 for example [40] keep quite well (less than 1% decomposition per year at 18.3 mc/mM, 0.1 mc/ml), and even solutions stored at room temperature are satisfactory for compounds which are not readily oxidised, and whose specific activity is moderate. For example DL-3-phenylalanine-1-C14 (10.8 mc/mM), glycine-C14 (U) (6.62 mc/mM), orotic acid-6-C14 (11.6 mc/mM), adenine-8-C14 sulphate (28.3 mc/mM) and thiamine-(thiazole-2-C14) hydrochloride (26.7 mc/mM), can all be stored at room temperature in aqueous solution at a radioactive concentration of 25 μc/ml with less than 2% decomposition per year [17]. However, until proven satisfactory for the compound under investigation, one must be cautious. Sodium acetate-1-C14 (37.3 mc/mM) undergoes 14% decomposition on storage in aqueous solution (25 μc/ml) during one year at room temperature, orotic acid-6-C14 (32.6 mc/mM) 25% decomposition per annum, and sodium pyruvate-1-C14 rapidly decomposes in aqueous solutions at room temperature [18]. In general, storage of solutions at low temperatures (−40 °C or below) or the addition of « protecting agents » (see below) are wise precautions.

SILVERSTEIN and BOYER[18] showed that the decomposition of pyruvate-C14 solutions at 25 °C is considerably reduced (from 84% to 4%) during 35 days storage, by the addition of benzene. The benzene had no protective effect in frozen solutions probably because it freezes out of solution. KORFF [61] also found pyruvate-C14 to be unstable in solution, but pyruvic acid -C14 stored at −30 °C is stable for at least 3 months.

Self-radiolysis of acetic acid-C14 in aqueous solution yielding non-ionic products (approx. 2.4%) is suggested [62] to explain the deviation from the second order law during a study of the kinetics of the esterification of ethanol with acetic acid-C14. As the radioactive concentration is only 15.45 μc/ml and the duration of the experiment only 170 hours, it would seem unlikely that radiation decomposition during the experiment is the complete explanation ; impurities in the acetic acid-C14 sample used is a more likely possibility, particularly if solutions had been kept for several days or weeks before use (*vide supra*).

TABLE 6. *Self-Decomposition of Compounds Labelled with Carbon-14*

Compound	Specific activity mc/mM	Storage condition	Temp. °C	Storage time months	Decomp. %	G(-M) *
AMINO ACIDS (Specifically labelled)						
DL-Alanine-1-C14	4.4	Solid	20	36	N.D.	—
DL-Alanine-1-C14	21.7	Freeze-dried solid under N_2	20	12	1	0.5
2-Amino*iso*butyric acid-1-C14	6.4	Freeze-dried solid under N_2 or *in vacuo*	20	36	N.D.	—
2-Amino*iso*butyric acid-1-C14	14.6		20	12	1	0.7
L-Citrulline-(*carboxyl*-C14)	22	Solid *in vacuo*	20	17	N.D.	—
DL-Cystine-3-C14 hydrochloride	18.7	Freeze-dried solid *in vacuo*	20	23	1	0.3
DL-3 (3,4-Dihydroxyphenyl)-alanine-2-C14	5.7	Solid	20	23	2	1.8
Folic acid-2-C14	31.4	Freeze-dried solid under N_2	—40	6	N.D.	—
Glycine-1-C14	7.9	Freeze-dried solid under N_2	20	24	N.D.	—
DL-Histidine-2-C14	21.6	Freeze-dried solid *in vacuo*	—40	21	N.D.	—
DL-5-Hydroxytryptophan-(*methylene*-C14)	7.45	Freeze-dried solid *in vacuo*	—40	32	N.D.	—
	2.34		—40	32	1	1.4
DL-Leucine-1-C14	36.4	Freeze-dried solid under N_2	20	2	1	1.7
DL-Lysine-1-C14	6.58	Freeze-dried solid *in vacuo*	20	22	4	3.4
DL-3-Phenyl(alanine-1-C14)	21	Freeze-dried solid under N_2	20	12	2	1.0
DL-3-Phenyl(alanine-2-C14)	4.6	Solid	20	65	3	1.2
DL-3-Phenyl(alanine-2-C14)	4.5	Freeze-dried solid *in vacuo*	20	47	N.D.	—
DL-3 (2-Thienyl) alanine-1-C14	0.78	Solid	20	50	1	3.4
DL-Tryptophan-(*benzene ring*-C14(U))	3.81	Freeze-dried solid under N_2	20	18	N.D.	—
DL-Tryptophan-(*methylene*-C14)	32.5	Freeze-dried solid under N_2	—40	6	N.D.	—
DL-Tryptophan-(*methylene*-C14)	32.5	Freeze-dried solid under N_2	—40	13	1	0.3
DL-Serine-3-C14	5.23	Freeze-dried solid *in vacuo*	20	36	N.D.	—
DL-Tyrosine-2-C14	4.2	Freeze-dried solid in air	20	63	N.D.	—

TABLE 6. (*continued*)

Compound	Specific activity mc/mM	Storage condition	Temp. °C	Storage time months	Decomp. %	G(-M) *
DL-Tyrosine-2-C14	15.8	Freeze-dried solid under N_2	20	12	N.D.	—
DL-Valine-1-C14	4.8	Freeze-dried solid *in vacuo*	20	33	N.D.	—
DL-Valine-4-C14	1.53	Solid under air	20	96	2	1.4
ALIPHATIC COMPOUNDS						
Acetic anhydride-1-C14	32.2	Liquid ° *in vacuo*	20	2	2	3.7
Acetone-1,3-C14	19.5	Liquid ° *in vacuo*	20	14	N.D.	—
Acetone-2-C14	5.4	Liquid ° *in vacuo*	20	29	3	2.3
Acetonitrile-2-C14	3.8	Liquid ° *in vacuo*	20	36	N.D.	—
Acetylene-C14(U)	7	Gas *in vacuo*	20	13	1	1.4
Acetyl bromide-1-C14	2.77	Liquid *in vacuo*	−40	12	10	39
Adipic acid-1,6-C14	5.8	Solid under air	20	31	3	2.0
Bromoacetic acid-1-C14	2.36	Sealed tube under air	20	36	N.D.	—
Bromoacetic acid-2-C14	4.87	Sealed tube under air	20	23	N.D.	—
Carbon tetrachloride-C14	7.2	Liquid ° *in vacuo*	20	3	N.D.	—
Cetane-1-C14	4.45	Benzene *in vacuo*	20	37	15	12
(*n*-hexadecane-1-C14)	10.7	Benzene *in vacuo*	20	3	2	7.6
Cetyl alcohol-1-C14	6.19	Sealed tube under air	20	43	4	1.9
Chloroacetic acid-1-C14	0.47	Solid in air	20	36	N.D.	—
Chloroacetic acid-1-C14	1.64	Solid in air	20	36	3	6.3
Choline chloride-(*methyl*-C14)	5.7	On paper	−80	11	N.D.	—
Choline chloride-(*methyl*-C14)	37.6	On paper	−80	18	N.D.	—
Creatinine-1-C14 hydrochloride	2.55	Freeze-dried *in vacuo*	20	22	1	2.1
*Cyclo*hexane-C14(U)	5.8	Liquid *in vacuo*	20	75	N.D.	—
Cyclohexane-1-carboxylic acid-(*carboxyl*-C14) Na salt	1.2	Freeze-dried *in vacuo*	20	75	N.D.	—
*trans-Cyclo*hexane-1,2-diamine tetracetic-2-C14 acid	7.69	Solid under air	20	60	5	1.4
Decamethonium bromide-(*methyl*-C14)	2.11	Solid in air	20	53	10	11.0
	5.03	Solid in air	20	12	1	2.1
n-Decane-1-C14	3.3	Liquid under air	20	35	N.D.	—
n-Decanoic acid-1-C14	3.5	Solid under N_2	20	24	N.D.	—
Dieldrin-C14	20.7	Benzene *in vacuo*	20	33	N.D.	—
Diethyl malonate-1-C14	1.5	Liquid under air	20	25	7	24
Diethyl malonate-1-C14	4.7	Liquid *in vacuo*	−40	11	N.D.	—
Diethyl malonate-2-C14	6.06	Liquid under air	20	42	14	7.3

TABLE 6. (*continued*)

Compound	Specific activity mc/mM	Storage condition	Temp. °C	Storage time months	Decomp. %	G(-M) *
Diethyl malonate-2-C14	4.36	Liquid *in vacuo*	−40	11	N.D.	—
N,N-Dimethyl (cetyl-1-C14) amine	4.5	Benzene *in vacuo*	20	34	3	2.3
Ethyl acetate-1-C14	10.2	Liquid ° in air	20	36	5	1.7
Ethylene diamine-C14(U) HCl	4.8	Solid *in vacuo*	20	24	N.D.	—
Ethylene diamine tetra-(aceticacid-2-C14) sodium salt	4.2	Solid in air	20	48	N.D.	—
	25	Solid in air	20	49	25	2.8
Ethyl iodide-1-C14	5.6	Liquid *in vacuo*	−40	18	4	4.8
Ethyl iodide-2-C14	4.28	Liquid *in vacuo*	−40	33	3	4.4
Fumaric acid-1,4-C14	4.9	Solid in air	20	11	2	4,5
Fumaric acid-1,4-C14	23.0	Solid in air	20	11	2	1.0
Fumaric acid-2,3-C14	6.15	Solid in air	20	22	N.D.	—
Glycerol-1-C14	1.6	Liquid *in vacuo*	−40	24	3	9.8
Glyceryl tri(stearate-1-C14)	14.5	Benzene *in vacuo*	20	55	N.D.	—
Glyceryl tri(stearate-2-C14)	1.36	Benzene *in vacuo*	−40	24	2	7.6
Glyceryl tri(oleate-1-C14)	9.8	Benzene *in vacuo*	20	16	N.D.	—
n-Hendecane-1-C14	2.5	Solid in air	20	42	N.D.	—
n-Hendecanoic acid-1-C14	2.35	Solid in air	20	90	4	2.1
Lauric acid-1-C14	2.6	Benzene *in vacuo*	20	22	1	2.1
Lauryl alcohol-1-C14	2.5	Benzene under N_2	20	24	N.D.	—
Lauryl alcohol-1-C14	10.7	Benzene under N_2	20	12	3	2.9
Linoleic acid-1-C14	24.7	Benzene under N_2	20	12	N.D.	—
Linoleic acid-C14(U)	39	Benzene *in vacuo*	20	24	2	0.3
Linolenic acid-C14(U)	139	Benzene under N_2	20	29	N.D.	—
Linolenic acid-C14(U)	139	Benzene *in vacuo*	20	16	8	0.4
Malathion-C14	2.2	Liquid in air	20	24	13	33
Methanol-C14	10.3	Liquid *in vacuo*	20	11	N.D.	—
Methyl cyanoacetate-2-C14	1.03	Liquid in air	20	42	8	2.3
Mevalonic lactone-1-C14	1.19	Benzene *in vacuo*	20	33	N.D.	—
Mevalonic lactone-2-C14	3.35	Benzene *in vacuo*	20	12	1	3.0
Maleic anhydride-2,3-C14	0.39	Solid *in vacuo*	20	21	N.D.	—
Maleic anhydride-2,3-C14	1.78	Solid *in vacuo*	20	21	5	16.3
Methylamine-C14 hydrochloride	2.65	Solid *in vacuo*	20	33	N.D.	—
Methyl bromoacetate-2-C14	2.9	Liquid in air	20	24	7	12.5
Methyl bromoacetate-2-C14	4.7	Liquid in air	20	12	N.D.	—
Methyl iodide-C14	15.2	Liquid *in vacuo*	−40	8	4	4.1
Methyl iodide-C14	25.2	Liquid *in vacuo*	−40	23	N.D.	—

TABLE 6. (*continued*)

Compound	Specific activity mc/mM	Storage condition	Temp. °C	Storage time months	Decomp. %	G(-M) *
n-Octadecane-1-C14	25.5	Solid under N_2	20	11	N.D.	—
Oleic acid-1-C14	24.6	Benzene *in vacuo*	20	3	1	0.2
Oleic acid-C14(U)	88	Benzene *in vacuo*	20	22	N.D.	—
Oxalic acid-C14(U)	14.7	Solid in air	20	12	1	0.7
Palmitic acid-C14(U)	76	Benzene *in vacuo*	20	31	N.D.	—
Palmitic acid-C14(U)	93	Benzene under N_2	20	25	3	0.2
Potassium cyanide-C14	0.43	Solid *in vacuo*	20	33	N.D.	—
Potassium thiocyanate-C14	4.4	Freeze-dried solid under air	20	24	3	3.5
*iso*Propyl iodide-1,3-C14	1.9	Liquid *in vacuo*	−40	36	N.D.	—
Sodium acetate-2-C14	10.0	Freeze-dried solid in air	20	6	N.D.	—
Sodium acetate-C14(U)	16.7	Freeze-dried solid under N_2	20	16	N.D.	—
Sodium acetate-C14(U)	7.1	Freeze-dried solid *in vacuo*	20	45	2	0.8
Sodium *n*-butyrate-1-C14	11.9	Solid *in vacuo*	20	30	10	3.5
Sodium *iso*butyrate-1-C14	2.4	Solid in air	20	84	20	13.5
Sodium *iso*caproate-1-C14	2.06	Solid in air	20	94	20	14
Sodium cyanide (alkaline)-C14	16.3	Solid *in vacuo*	20	24	2	0.6
Sodium cyanoacetate-2-C14	4.7	Solid in air	20	31	N.D.	—
Sodium formate-C14	6.1	Freeze-dried solid *in*	20	51	2	0.8
Sodium formate-C14	17.4	*vacuo*	20	12	N.D.	—
Sodium glyoxalate-1-C14	4.8	Solid under N_2	20	10	1	2.4
Sodium *n*-heptanoate-1-C14	2.6	Solid in air	20	88	20	11.8
Sodium *n*-hexanoate-1-C14	2.87	Solid in air	20	86	10	5.2
Sodium 2-ketoglutarate-5-C14	3.05	Solid in air	−40	24	10	17.7
Sodium 2-ketoglutarate-5-C14	6.93	Solid in air	−40	12	1	1.5
Sodium DL-lactate-2-C14	4.88	Solid *in vacuo*	−40	26	2	1.9
Sodium *n*-nonanoate-1-C14	1.94	Solid in air	20	36	N.D.	—
Sodium *n*-octanoate-1-C14	3.3	Solid in air	20	35	N.D	—
Sodium propionate-1-C14	5.6	Freeze-dried solid in air	20	60	3	1.1
Sodium propionate-2-C14	3.7	Freeze-dried solid under N_2	20	24	3	4.2
Sodium propionate-2-C14	4.3	Freeze-dried solid in *vacuo*	20	35	7	5.7
Sodium pyruvate-1-C14	16.0	Freeze-dried solid	−40	6	1	1.3
Sodium pyruvate-1-C14	5.0	under N_2	−40	9	1	2.7

TABLE 6. (*continued*)

Compound	Specific activity mc/mM	Storage condition	Temp. °C	Storage time months	Decomp. %	G(-M) *
Sodium pyruvate-2-C14	2.7	Freeze-dried solid *in vacuo*	−40	22	7	15
Sodium pyruvate-3-C14	2.1	Freeze-dried solid in *vacuo*	−40	24	5	13
Sodium pyruvate-C14(U)	2.1	Freeze-dried solid *in vacuo*	−40	22	N.D.	—
Sodium pyruvate-C14(U)	2.14	Freeze-dried solid under N_2	−40	8	2	13.6
Stearic acid-C14(U)	92	Benzene *in vacuo*	20	31	N.D.	—
Stearyl alcohol-1-C14	1.89	Benzene *in vacuo*	20	47	N.D.	—
Succinic acid-2,3-C14	13.9	Solid *in vacuo*	20	72	3	0.4
Succinic acid-2,3-C14	4.25	Solid in air	20	24	2	2.4
Succinyl *bis*(choline-C14 iodide)	8.6	Solid in air	20	19	2	1.4
DL-Tartaric acid-1,4-C14	3.4	Solid in air	20	24	N.D.	—
AROMATIC COMPOUNDS						
Acetyl salicyclic acid-(*carboxyl*-C14)	1.0	Solid in air	20	80	N.D.	—
DL-*nor*Adrenaline-(*carbinol*-C14) DL-bitartrate	10.9	Freeze-dried solid *in vacuo*	20	19	N.D.	—
Aniline-C14(U) sulphate	5.1	Solid under N_2	20	44	2	1.1
Aniline-C14(U) sulphate	5.1	Solid *in vacuo*	20	32	N.D.	—
Benzaldehyde-C14 (*carbonyl*-C14)	1.3	Sealed tube under air	20	28	4	13.6
1,2-Benzanthracene-9-C14	5.52	Benzene *in vacuo*	20	66	N.D.	—
Benzene-C14(U)	10.4	Liquid ° in *vacuo*	−40	36	N.D.	—
γ-Benzenehexachloride-C14(U)	9.4	Benzene *in vacuo*	20	36	N.D.	—
Benzoic acid-(*ring*-C14(U))	5.1	Solid in air	20	33	1	0.7
Benzylpenicillin-C14	24.7	Freeze-dried solid *in*	−40	6	2	1.6
(potassium salt)	24.7	*vacuo*	−40	9	12	7.0
Bromobenzene-C14(U)	2.7	Liquid in air	20	21	3	6.5
Chlorobenzene-C14(U)	6.9	Liquid in air	20	24	3	2.2
4-Chloro-2-methylphenoxy-(acetic-1-C14) acid	2.55	Solid under air	20	75	N.D.	—
'D.D.T.'-(*phenyl*-C14)	4.4	Benzene *in vacuo*	20	23	N.D.	—
1,2,3,4-Dibenzanthracene-9-C14	2.8	Benzene *in vacuo*	20	58	30	27
2,4-Dichlorophenoxy-(acetic-1-C14) acid	2.94	Benzene in *vacuo*	20	27	N.D.	—
	14.1	Benzene *in vacuo*	20	36	8	2.0
γ-2,3-Dichlorophenoxy-(butyric-1-C14) acid	12.6	Benzene *in vacuo*	20	61	N.D.	—
Diethyl stilboestrol-1-C14	10.9	Benzene *in vacuo*	20	32	N.D.	—

TABLE 6. (*continued*)

Compound	Specific activity mc/mM	Storage condition	Temp. °C	Storage time months	Decomp. %	G(-M) *
9,10-Dimethyl-1,2-benzanthracene-9-C14	9.3	Solid in air	0	4	2	6.8
	9.3	Solid in air	0	21	6	3.7
2,4-Dinitrochlorobenzene-C14(U)	6.84	Solid in air	20	24	4	3.0
1-Fluoro-2,4-dinitrobenzene-C14(U)	4.37	Liquid under N_2	20	15	N.D.	—
Naphthalene-1-C14	2.0	Solid under N_2	20	73	N.D.	—
1-Naphthoic acid-(*carboxyl*-C14)	5.8	Solid in air	20	36	N.D.	—
2-Naphthoic acid-(*carboxyl*-C14)	4.6	Solid in air	20	112	2	0.5
Nitrobenzene-C14(U)	2.4	Liquid under air	20	44	N.D.	—
2-Methyl-C14-naphthaquinone	1.63	Solid under air	20	43	3	5.4
2-Methyl-C14-naphthaquinone	1.63	Solid under air	−40	50	N.D.	—
Phenanthrene-9-C14	1.93	Solid in air	20	36	5	9.0
Phenol-C14(U)	1.5	Solid under air	−40	24	10	36
Terephthalic acid-(*carboxyl*-C14)	4.0	Solid in air	20	64	20	10.7
o-Toluic acid-(*carboxyl*-C14)	6.75	Solid in air	20	33	N.D.	—
p-Toluic acid-(*carboxyl*-C14)	2.27	Solid in air	20	26	1	2.1
m-Toluic acid-(*carboxyl*-C14)	1.84	Solid in air	20	66	2	2.1
HETEROCYCLIC COMPOUNDS						
Adenine-8-C14	31.3	Solid under N_2	20	12	1	0.3
D-Biotin-(*carbonyl*-C14)	2.65	Solid *in vacuo*	20	24	2	3.9
D-Biotin-(*carbonyl*-C14)	32.4	Solid *in vacuo*	−40	20	1	0.2
Guanine-8-C14 sulphate	15.4	Solid in air	20	24	N.D.	—
Hypoxanthine-8-C14	12.2	Solid under N_2	20	9	1	1.1
5-Hydroxytryptamine-3'-C14 creatinine sulphate	11.4	Solid *in vacuo*	−40	12	N.D.	—
	32.0	Solid under N_2	20	12	1	0.4
Indole (acetic-2-C14) acid	5.3	Solid in air	20	24	3	2.9
3-Indolyl (acetonitrile-1-C14)	11.6	Benzene *in vacuo*	20	37	1	0.3
Nicotinamide-(*carbonyl*-C14)	10.7	Solid in air	20	24	N.D.	—
Nicotinic acid-(*carboxyl*-C14)	10.7	Solid *in vacuo*	20	72	N.D.	—
*iso*Nicotinic hydrazide-(*carbonyl*-C14)	9.8	Solid *in vacuo*	−40	31	N.D.	—

TABLE 6. (*continued*)

Compound	Specific activity mc/mM	Storage condition	Temp. °C	Storage time months	Decomp. %	G(-M) *
Orotic acid-6-C14	32.6	Solid in air	20	12	1	0.3
Thiamine-2-C14 hydrochloride	26.7	Solid under N_2	20	25	1	0.2
Thymidine-2-C14	18.3	Solid *in vacuo*	−40	60	1	0.1
Thymine-2-C14	9.5	Solid under air	20	47	2	0.5
Uracil-2-C14	37.5	Solid under N_2	20	12	2	0.5
Uric acid-2-C14	8.23	Solid under N_2	20	24	3	1.4
STEROIDS						
Cholesterol-4-C14	13.7	Benzene *in vacuo*	20	20	1	0.5
Cholesterol-26-C14	24.0	Benzene *in vacuo*	20	37	1	0.2
Cholestenone-4-C14	19.3	Benzene *in vacuo*	20	64	N.D.	—
Cholesteryl linoleate-1-C14	10.3	Benzene *in vacuo*	20	12	40	51
Cholesteryl linoleate-1-C14	10.9	Benzene under nitrogen	20	12	7	6.6
Cholesteryl palmitate-1-C14	11.0	Benzene *in vacuo*	20	18	N.D.	—
Cholesteryl-4-C14 palmitate	21.2	Benzene *in vacuo*	20	24	N.D.	—
Cortisol-4-C14	22.3	Benzene under N_2	20	9	3	1.8
Cortisol-4-C14	22.3	Benzene/2% EtOH under nitrogen	20	12	4	1.8
Cortisol-4-C14	22.3	Benzene/5% MeOH under nitrogen	20	12	7	3.3
Cortisol-4-C14	28.6	Benzene/10% MeOH under nitrogen	20**	12	34	15
Cortisol-4-C14	29.2	Benzene/5% MeOH under nitrogen	−40	12	8	2.9
Cortisol-4-C14	29.2	EtOH under N_2	0	12	13	5
Cortisol-4-C14	29.2	Benzene/5%MeOH under nitrogen	20	12	22	8.7
Cortisol-4-C14	29.2	Benzene/10%MeOH	0	12	27	11.1
Cortisol-4-C14	29.2	under nitrogen	20	12	32	13.6
Cortisone-4-C14	22.6	Benzene *in vacuo*	20	12	7	3.2
Cortisone-4-C14	25.0	Benzene *in vacuo*	20	48	N.D.	—
Cortisone-4-C14 acetate	17.1	Benzene *in vacuo*	20	46	N.D.	—
Progesterone-4-C14	26.1	Benzene *in vacuo*	20	18	25	7.5
Pregnenolone-4-C14	19.8	Benzene *in vacuo*	20	12	2	1.0
Testosterone-4-C14 propionate	10.2	Benzene *in vacuo*	20	69	N.D.	—

* = Calculated from the equation [3] assuming '*f*' is unity
** = Sample left in the laboratory in occasional sunlight
° = Or as vapour
N.D. = No detectable decomposition

Mizon, Boulanger and Osteux[63] draw attention to the possible decomposition of amino acids during the actual purification procedures. The carbon-14 labelled acids, arginine, ornithine, lysine, aspartic and glutamic acids, proline and pipecolic acid, may undergo from 1 — 5% decomposition if left during 8 days at room temperature in the presence of air. It is necessary to guard against such chemical decomposition effects.

Examination of Table 6 shows that the rate of decomposition of cortisol-4-C14 stored in benzene solution, is accelerated by the addition of 5% or more of methanol [17]. However, a few percent alcohol can often have a protective action when added to aqueous solutions of labelled compounds [26, 28, 30]. The protective effect of small amounts of ethanol [28] on solutions of amino acids-C14 (U) at high specific activity is seen from Table 7.

If ethanol is unacceptable to the user of the labelled compound, the stability of the amino acids in aqueous solution is increased by storage at −40 °C.

In general phenylalanine-C14(U) is the most sensitive to self-radiolysis of the carbon-14 amino acids; it has, also, the highest molar specific activity for the same isotopic abundance.

Reducing sugars are amongst the more susceptible of labelled compounds to the influence of their own radiation. Two reasons for this are the effect of oxidising agents produced in solution, and the difficulty of obtaining anhydrous sugars for storage in the dry state. Some results are recorded in Table 8.

The best method of storage for the sugars will vary with the user's requirement, but the following might be generally recommended for glucose-C14 :

(i) Specific activity less than 0.1 mc/mM
Crystalline, anhydrous, with precautions to ensure samples remain in a dry atmosphere.

(ii) Specific activity 0.1-3 mc/mM
Freeze-dried, sealed under vacuum, and spread out as much as possible over the surface of the tube (i.e. freeze-dried from as large a volume as possible)

(iii) Specific activity above 3 mc/mM
Storage on paper (sealed under vacuum) is probably the best method, but this may sometimes be found inconvenient. Aqueous ethanolic solutions are a possible alternative, but this is still under investigation [17].

The labelled ribonucleosides-C14(U) are best stored in aqueous solution at −40 °C (or below); some results are shown in Table 9.

If storage at room temperature is essential, the rate of decomposition of the ribonucleosides-C14(U) may be reduced by the addition of a few percent ethanol, but as with other carbon-14 labelled compounds the ethanol does not give increased stability at −40 °C [28]. Current work at the Radiochemical Centre indicates that the deoxyribonucleosides have similar stability to the ribonucleosides provided they are carefully purified from chemical impurities. No information is available concerning the stability of the nucleotides labelled with carbon-14.

TABLE 7. *Self-Radiolysis of Amino Acids Uniformly Labelled with Carbon-14*

Amino acid-C14(U)	Specific activity mc/mM	Storage conditions *	Temp. °C	Time weeks	Decomp. %	*f* %	G(-M) **
L-Alanine	15	Freeze-dried, in air	20	104	1	27	1.5
L-Alanine	85	Water under N_2	25	31	3.0	100	0.6
L-Arginine	35	Freeze-dried, in air	20	108	22	45	7.8
L-Arginine	193	Water under N_2	25	33	21.7	100	2.1
L-Aspartic acid	106	Water under N_2	25	30	2.8	100	0.5
L-Glutamic acid	27	Freeze-dried, in air	20	100	16	42	8.4
L-Glutamic acid	148	Water ° under N_2	25	35	13.4	100	1.5
Glycine	67	Water under N_2	25	29	1.4	100	0.4
L-Leucine	30	Freeze-dried, in air	20	117	3	5	9.7
L-Leucine	170	Water under N_2	25	31	17.8	100	2.0
L-*iso*Leucine	174	Water under N_2	25	37	11.7	100	1.0
L-Phenylalanine	282	Water under N_2	25	39	39.9	100	2.5
L-Phenylalanine	282	Water under N_2	20	8	8.4	100	2.1
L-Phenylalanine	282	Water under N_2	2	8	6.1	100	1.5
L-Phenylalanine	282	Water under N_2	−20	8	1.0	100	0.2
L-Phenylalanine	282	Water/3% EtOH under N_2	20	8	<0.3	100	<0.1
L-Phenylalanine	282	Water/3% EtOH under N_2	−20	8	<0.3	100	<0.1
L-Serine	17	Freeze-dried, in air	20	95	20	63	11.4
L-Serine	87	Water under N_2	25	33	5.2	100	1.0
L-Threonine	26	Freeze-dried, in air	20	87	8	32	6.2
L-Threonine	133	Water under N_2	25	38	8.5	100	0.9
L-Tyrosine	240	Water under N_2	25	33	11.8	100	0.9
L-Valine	26	Freeze-dried, in air	20	95	3	26	2.6

* = Aqueous solutions autoclaved for 30 minutes at 120 °C
° = Cannot be autoclaved without decomposition — sterile filtered
** = Calculated from the equation (3).

Decomposition by free radical reactions can sometimes proceed at great speed. Thus *iso*butene-C14, even at 2.5 mc/mM, polymerises within a few minutes on freezing in liquid nitrogen. Similarly, acrylonitrile-1-C14 at 1.55 mc/mM is largely converted into a solid polymer on standing at room temperature in the dark overnight [17]. N-(M*ethyl*-C14)-N-nitroso-*p*-toluenesulphonic acid (11 mc/mM) and N-ethyl (maleimide-2,3-C14) (2.6 mc/mM) are two other compounds which undergo considerable decomposition during a few weeks even at −40 °C [17].

TABLE 8. *Self-Radiolysis of Carbohydrates Uniformly Labelled with Carbon-14*

Compound	Specific activity mc/mM	Storage conditions °	Temp. °C	Time weeks	Decomp. %	*f* %	G(-M)**
D-Fructose	86	On paper *in vacuo*	−40	69	1.5	0.3*	45
D-Fructose	26	Freeze-dried *in vacuo*	20	30	8.0	40	14
D-Fructose	26	Freeze-dried *in vacuo*	−80	30	5.9	40	10
D-Glucose	42	Freeze-dried *in vacuo*	20	34	13.1	10	53
D-Glucose	42	Freeze-dried *in vacuo*	−80	34	3.6	10	14
D-Glucose	42	On paper *in vacuo*	20	34	1.2	0.05*	900
D-Glucose	42	On paper *in vacuo*	−80	34	0.7	0.05*	526
D-Glucose	42	Crystalline solid *in*	20	34	9.7	100	3.8
D-Glucose	42	*vacuo*	−80	34	5.7	100	2.2
D-Glucose	4.8	Water (0.5 mc/ml)	2	40	2.0	100	5.6
D-Glucose	4.8	Water (0.5 mc/ml)	−40	40	1.1	100	3.1
D-Glucose	80	Water (0.5 mc/ml)	2	40	15.4	100	2.8
D-Glucose	80	Water (0.5 mc/ml)	−40	40	7.0	100	1.2
D-Glucose	80	Water (0.05 mc/ml)	2	40	12.4	100	2.2
D-Glucose	80	Water (0.05 mc/ml)	−40	40	5.1	100	0.9
Sucrose	149	Freeze-dried *in vacuo*	20	88	16.4	16	4.6
Sucrose	149	Freeze-dried *in vacuo*	−80	88	15.1	16	4.2
Sucrose	149	On paper in air	20	88	15.7	0.3*	234
Sucrose	149	On paper in air	−80	88	4.9	0.3*	68
Sucrose	149	On paper *in vacuo*	20	88	2.4	0.3*	33
Sucrose	149	On paper *in vacuo*	−80	88	1.8	0.3*	25
Sucrose	171	Water (0.05 mc/ml)	2	40	16.8	100	1.4
Sucrose	171	Water (0.5 mc/ml)	2	40	27.7	100	2.5

* = Absorbed energy by the sugar only, i.e. excluding the paper from the 'system'.
** = Calculated from the equation [3].
° = Solutions sterilised by autoclaving at 120° for 30 minutes.

(e) *Tritium Compounds*

The almost complete absorption of the beta radiation energy and the very high specific activities which can be attained has made the control of self-radiolysis for tritium compounds much more difficult than for compounds labelled with other radioisotopes. The decomposition of tritium compounds has consequently been studied in rather more detail than other labelled compounds (see Table 3).

Fairly comprehensive reviews concerning the decomposition of organic compounds labelled with tritium have already been published ([13, 19-26]) and it is perhaps only necessary for this review to 'highlight' some of the observations of general importance. These are:

TABLE 9. *Self-Radiolysis of Nucleosides Uniformly Labelled with Carbon-14*

Compound	Specific activity mc/mM	Storage condition °	Temp. °C	Storage time weeks	Decomp. %	G(-M) *
Adenosine-C14(U)	307	Water *in vacuo*	37	17	3.5	0.4
Adenosine-C14(U)	307	Water *in vacuo*	−40	17	1.7	0.2
Cytidine-C14(U)	256	Water *in vacuo*	37	17	5.5	0.7
Cytidine-C14(U)	256	Water *in vacuo*	−40	17	1.3	0.2
Guanosine-C14(U)	281	Water *in vacuo*	37	19	7.6	0.8
Guanosine-C14(U)	281	Water *in vacuo*	−40	19	0.8	0.1
Uridine-C14(U)	251	Water *in vacuo*	37	22	9.0	0.9
Uridine-C14(U)	251	Water *in vacuo*	−40	22	2.3	0.2

° = Solutions sterilised by autoclaving for 30 minutes at 120 °C; activity concentration 0.5 mc/ml.
* = Calculated from the equation [3] with *'f'* as unity.

(i) The rate of decomposition may vary markedly between different preparations of the same compound stored apparently under identical conditions at the same molar specific activity.

(ii) There is normally little variation between the observed decomposition of samples of the compound (dispensed into ampoules at one time) prepared from a single batch and stored under identical conditions.

(iii) Racemization of optically active amino acids may occur on storing aqueous solutions of tritiated amino acids at + 2 °C [26]. This has not yet been observed for carbon-14 or sulphur-35 labelled amino acids stored in aqueous solution.

(iv) Self-radiolysis of unsaturated compounds dissolved in benzene solution have not yet been observed to result in any significant *cis-trans* isomerism. Less than 5% *trans*-isomer is found during the storage of oleic acid-9, 10-T or glyceryl-2-T trioleate (see Table 10), although about 10^5 rads of radiation energy were absorbed by the solutions. It is interesting to note that benzene solutions of some unsaturated compounds have been observed to undergo *cis-trans* isomerism when irradiated externally at comparable radiation doses [65, 66].

(v) A slight variation in the conditions of storage can sometimes markedly affect the G(-M) value. Thus, slowly frozen solutions of tritiated thymidine, for example, undergo a more rapid decomposition than solutions stored at + 2 °C [40–45].

TABLE 10. *Self-Radiolysis of Some Tritium Labelled Compounds*

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M)*
Adenine-2,8-T	2,900	Water (1 mc/ml)	2	9	22	1.0
Adenosine-T(G)-5′-monophosphate (lithium salt)	644	Water (0.5 mc/ml)	2	3	N.D.	—
S-Adenosylmethionine-(*methyl*-T)	200	Aq. sulphuric acid	20	0.17	10	350
	1,580	at pH 4 (1 mc/ml)	20	0.23	10	32
S-Adenosylmethionine-(*methyl*-T)	200	Aq. sulphuric acid at	−80	1.5	8	30
	1,580	pH 4 (1 mc/ml)	−80	1.5	8	3.7
S-Adenosylmethionine-(*methyl*-T)	1,580	Aq. sulphuric acid at pH 4 (1 mc/ml)	−196	2	7	2.4
d(+)-Aldosterone-1,2-T	1,940	Benzene + 10%	2	6	<5	<0.5
d(+)-Aldosterone-1,2-T	10,400	EtOH (0.2 mc/ml)	2	6.5	<5	<0.1
Benzyl-T(G) penicillin	189	Freeze-dried in *vacuo*	−40	5	5	5.7
Benzyl-T(G) penicillin	189	Water (1 mc/ml)	2	5	90	260
3,4-Benzpyrene-T(G)	1,760	Benzene (6.7 mc/ml)	−40	13	4	0.2
Cholesterol-1α-T	506	Benzene (1.4 mc/ml)	20	6	2	0.7
Cholesterol-1α-T	7,150	Benzene (1 mc/ml)	20	6	8	0.2
Cholesterol-7α-T	3,450	Benzene (10 mc/ml)	20	4.5	N.D.	—
Cytidine-T(G)	2,300	Water (1 mc/ml)	2	13	20	0.8
Dehydro*epi*androsterone-7α-T	18,300	Benzene (2.2 mc/ml)	20	13	2	0.01
Dehydro*epi*androsterone-7α-T acetate	1,460	Benzene (1.5 mc/ml)	20	15	6	0.3
Deoxycytidine-5-T	8,300	Water (1 mc/ml)	2	8	25	0.5
Deoxyuridine-5,6-T	5,160	Water (1 mc/ml)	2	9	35	1.0
Dimethylaniline-T(G)	95	Liquid under air	2	32	N.D.	—
9,10-Dimethyl-1,2-benzanthracene-T(G)	189	Freeze-dried solid *in*	−80	5	N.D.	—
	15,100	*vacuo*	−80	4.5	40	0.8
1-Fluoro-2,4-dinitro-benzene-3,5,6-T	10,400	Benzene (34 mc/ml) in the dark	20	8	N.D.	—
5-Fluorouracil-6-T	624	Water (1 mc/ml)	2	12	<5	<0.8
5-Fluorouracil-6-T	624	Water (1 mc/ml)	−196	12	<2	<0.3
Glycerol-2-T	71	Liquid in air	2	33	<2	<1.1
Glyceryl-2-T trioleate	104	Benzene (4.4 mc/ml)	20	31	<2	<0.7
Glyceryl tri(stearate-9,10-T)	4,300	Benzene (30 mc/ml)	20	9	8	0.2
DL-Leucine-4,5-T	785	Water (1 mc/ml)	2	11	13	1.8
DL-Leucine-4,5-T	23,000	Water (1 mc/ml)	2	3	10	0.2
D-Leucine-4,5-T	500	Water (1 mc/ml)	2	5	5	2.2
D-Leucine-4,5-T	16,100	Water (1 mc/ml)	2	5	10	0.1
L-Leucine-4,5-T	23,000	Water (1 mc/ml)	2	5	10	0.1
L-Methionine-(*methyl*-T)**	1,610	Water (2.9 mc/ml)	2	3.5	15	3.1
L-Methionine-(*methyl*-T)	1,610	Water (1 mc/ml)	2	3.5	13	2.7

TABLE 10. (*continued*)

Compound	Specific activity mc/mM	Storage conditions	Temp. °C	Storage time months	Decomp. %	G(-M) *
L-Methionine-(*methyl*-T)	1,610	Water + 1% sodium formate(2.9 mc/ml)	2	3.5	15	3.1
L-Methionine-(*methyl*-T)	1,610	Water + 0.01% sodium formate (2.9 mc/ml)	2	3.5	15	3.1
L-Methionine-(*methyl*-T)	1,610	Water + 0.1% ethanol (2.9 mc/ml)	2	3.5	15	3.1
Nicotine-T(G)	761	Liquid *in vacuo*	−40	14	45	6.3
Oleic acid-9,10-T	730	Benzene (2.5 mc/ml)	20	9	8	1.4
Oleic acid-9,10-T	2,480	Benzene (2.5 mc/ml)	20	5	4	0.3
Orotic acid-5-T	4,600	Water (1 mc/ml)	2	8	71***	—
Palmitic acid-9,10-T	850	Benzene (1.9 mc/ml)	20	8.5	5	0.8
L-Phenylalanine-(*ring*-4-T)	1,000	Water (1 mc/ml)	2	5	5	1.1
L-Phenylalanine-(*ring*-4-T)	2,000	Water (1 mc/ml)	2	12	25	1.4
L-Phenylalanine-(*ring*-4-T)	9,600	Water (1 mc/ml)	2	3.5	10	0.3
β-Propiolactone-T	202	Liquid + 5% ether	2	5	60	97
Succinic acid-2,3-T	330	Solid in air	−40	15	10	2.4
Succinic acid-2,3-T	649	Solid in air	2	14	16	2.2
Thymine-T(G)	12,200	Water (1 mc/ml)	2	4.5	18	0.4
DL-Tryptophan-T(G)	1,460	Water (1.2 mc/ml)	−40	15	15	0.9
L-Tyrosine-3,5-T	1,300	Water (1 mc/ml)	2	6	15	2.2
Uracil-5,6-T	1,750	Water (1 mc/ml)	2	5	12	1.6
Uridine-T(G)	1,760	Water (5.4 mc/ml) + 0.01% sodium formate	2	3.25	15	3.0
Uridine-5-T-5′monophosphate (ammonium salt)	572	Water (1 mc/ml)	2	3	N.D.	—
	7,320	Water (1 mc/ml)	2	3	N.D.	—

Benzene solutions sealed under *vacuo*
N.D. = No detectable decomposition
* = Calculated from the equation [2]
** = No racemization detected in stored solutions of this compound
*** = Detailed analysis showed 66% labile tritium and 5% non-volatile radiochemical impurity. Only 30-33% of orotic acid remained as determined by UV-light absorption measurements.

(vi) Some compounds at high specific activity (for example, orotic acid-5-T) can be stored in aqueous solution, apparently without significant radiolysis. However, a more detailed analysis indicates that the molecular structure is destroyed, giving rise to 'labile' tritium (which exchanges to form tritiated water) and unlabelled chemical impurities. This possibility should always be borne in mind when analysing tritium compounds.

OSINSKI[52] has recently studied the comparative decomposition rates for nine tritiated steroids stored in several solvents. The sensitivity to self-radiolysis increases as the number of oxygen atoms in the steroid molecule is increased.

Some recent results obtained at the Radiochemical Centre for a miscellany of tritiated compounds are shown in Table 10.

(f) *Selenium-75 Compounds*

Compounds labelled with the gamma emitter selenium-75 would not be expected to undergo serious self-radiolysis. L-Selenomethionine-Se75, the selenium analogue of the naturally occurring amino acid methionine, is the only compound studied at present. Storage of this compound at 400 mc/mM. in aqueous solution at $-30\,^{\circ}C$ and a radioactive concentration of 8 mc/ml, or at room temperature at 1 mc/ml, results in no detectable decomposition over a period of six months [17].

(g) *Iodine-125 and Iodine-131 Compounds*

The number of systematic investigations concerning the stability of compounds labelled with radioactive iodine is even less than for those labelled with most other isotopes. Many of these compounds are used *in vivo* and under these circumstances the important parameter to measure has usually been taken to be the liberation of free iodide rather than the overall drop in radiochemical purity [67, 71]. Clearly there is good reason behind this viewpoint, but it can be misleading; a faulty diagnosis due to the use of an impure radiochemical can be at least as damaging to a patient as an unwanted radiological dose to his thyroid.

Table 11 gives some results obtained on the decomposition of these compounds at room temperature [17].

As with compounds labelled with other radioisotopes, the decomposition rates depend on the exact storage conditions; in addition, the problem of reliable analysis is met in an acute form for the iodothyronines.

Although the decomposition rates quoted (Table 11) are in some cases quite high, they do not represent examples of exceptionally labile compounds; the case of triiodothyronine-I131 reported in Table 11 has a G(-M) value of less than 0.06.

Compounds labelled with iodine-125 are more stable than those correspondingly labelled with iodine-131 as would be expected from the lack of beta-emission from I-125.

Fluorinated xenon compounds have been isolated during the decay of I-131 in iodine pentafluoride-I131 [68].

Radionuclides of iodine are also used to « tag » molecules such as proteins which cannot be conveniently labelled by the incorporation of an appropriate isotope. In these cases it is not possible to describe a « radiochemical impurity »

TABLE 11. *Self-Radiolysis of Some Compounds Labelled with I-125 and I-131*

Compound	Specific activity mc/mM	Storage conditions	Radioactive concentration mc/ml	Storage time (days)	Impurity % Total Iodide	
4-Iodoantipyrine-I125	126	Neutral aq. soln.	0.075	104	N.D.	N.D.
4-Iodoantipyrine-I131	950	Neutral aq. soln.	1.5	9	—	15
5-Iodo-2′-deoxyuridine-I125	920	Aqueous solution	0.126	30	—	1
5-Iodo-2′-deoxyuridine-I131	280	Aqueous solution	0.79	14	9	—
5-Iodo-2′-deoxyuridine-I131	5,800	Aqueous solution	0.46	14	10	—
o-Iodohippuric acid-I125 (sodium salt)	3.4	Aqueous solution	0.28	92	N.D.	N.D.
o-Iodohippuric acid-I131 (sodium salt)	25	Aqueous solution	3.75	8	—	1
Triiodothyronine-I125	3,250	50% v/v aqueous propylene glycol	0.17	40	—	1.2
Triiodothyronine-I131	25,200	50% v/v aqueous propylene glycol	0.62	28	15	4
Thyroxine-I125	3,900	50% v/v aqueous propylene glycol	0.19	40	—	1.5
Thyroxine-I131	41,500	50% v/v aqueous propylene glycol	0.48	21	12	7

N.D. = No detectable decomposition

in the same way as one can do for 'normally' labelled compounds. One can only speak of a certain percentage of the iodinated compound behaving in an identical manner to the compound for which it is serving as a tracer *under given test conditions*. For example, one could measure the stability of iodinated insulin. One value would be obtained for its behaviour *in vivo* and quite another might be obtained by measurement of the amount of activity bound to antibody — a parameter of some importance to those wishing to use the labelled protein for radio-immunoassay. However, many of the general remedial measures can be applied equally well for minimising the decomposition of such iodinated materials by storage at very low temperatures for example, and by the addition of an inactive protein as a 'protective agent'.

(h) *Cobalt-57 and Cobalt-58 Compounds*

The only compound labelled with these radioisotopes which has been studied to any extent is cyanocobalamin (vitamin-B_{12}), whose instability was first

reported by SMITH[37]. A further series of observations on the decomposition of labelled cyanocobalamins in aqueous solution was later reported by ROSENBLUM[38].

For cyanocobalamin labelled with cobalt-58 at moderate specific activity, the best method of storage appears to be as a thin film of freeze-dried solid, as illustrated by the results[17] shown in Table 12.

TABLE 12. *Self-Radiolysis of Cyanocobalamin-Co58*

Compound	Specific activity curies/mM	Storage conditions	Temp. °C	Storage time weeks	Decomposition %
Cyanocobalamin-Co58	10	Aqueous solution	2	10	45
Cyanocobalamin-Co58	10	Aqueous solution	−40	10	15
Cyanocobalamin-Co58	10	Freeze-dried solid	2	10	None detectable

Cyanocobalamin labelled with cobalt-57 is not so stable as the compound labelled with cobalt-58 when freeze-dried and neither is it stable when dissolved in aqueous solution. However, the addition of 0.9% benzyl alcohol to the solution considerably reduces the rate of decomposition even when the cyanocobalamin is at high specific activity. Benzyl alcohol can also be used for stabilising solutions of the compound labelled with cobalt-58 and these results are summarised in Table 13.

The main product from the self-irradiation decomposition of cyanocobalamin-Co57 (or Co58) has been identified as hydroxocobalamin[17].

TABLE 13. *Protective Effect of Benzyl Alcohol on Solutions of Labelled Cyanocobalamins*

Compound	Specific activity curies/mM	Storage conditions	Temp. °C	Storage time weeks	Decomposition %
Cyanocobalamin-Co57	390	Freeze-dried * solid	2	12	39
Cyanocobalamin-Co57	390	Aqueous solution + 0.9% benzyl alcohol	2	12	None detectable
Cyanocobalamin-Co58	400	Aqueous solution + 0.9% benzyl alcohol	2	11	None detectable

* = included for comparison

Care must be taken with cyanocobalamin to ensure ordinary chemical stability by storing the compound in a cool (or cold) dark place free from noxious contaminants.

6. Control of self-irradiation decomposition

Fortunately the decomposition of radioactive compounds by self-irradiation can be controlled and minimised to a tolerable level for most uses of these compounds. As already discussed, the problem is usually quite small for compounds labelled with chlorine-36, phosphorus-32, sulphur-35 or with pure gamma emitters.

There are three main methods for the control of self-radiolysis. These are :

(a) Dispersion of the active molecules

(b) Reduction of the temperature of storage

(c) Scavenging of the reactive species such as free radicals.

In practice a combination of all three methods is often used.

(a) *Dispersion of the active molecules*

Homogeneous dilution of the labelled material with the same unlabelled compound (inactive carrier) serves to disperse the active molecules but also reduces the specific activity of the compound, which is often unacceptable on other grounds. It is a wise precaution to dilute the radioactive compound to the lowest convenient molar specific activity for use.

From Tables 6, 7, 8 and 9 it is observed that the dispersal of molecules labelled with carbon-14 on paper, in aqueous solution, as freeze-dried solids or dissolved in organic solvents, are all satisfactory methods for reducing their rate of decomposition.

For tritium compounds, dispersal of the compound as a thin film, on paper and other supports is much less effective in reducing their decomposition rate [19, 23, 29, 69]. Using such supporting materials as charcoal, benzanthracene, silica gel, iron oxides or cellulose powder [23], or sand [29], offers only more complications to the research worker for recovering the labelled compound ready for use in return for only a modest reduction in the G(-M) value. The use of clathrates has been tried by Guarino and his colleagues [19,69] who recognised, firstly, the difficulty in preparing a suitable clathrate and secondly the problem of recovering a chemically and radiochemically pure compound from the clathrate. Labelled compounds, although perhaps representing only a small fraction of the expenditure on a research project, are valuable and their preparation may represent many months work ; recoveries of such compounds from any method of storage must be high.

In general a solution of the radioactive compound in a suitable solvent is preferred to dispersal on foreign solid supporting materials.

(b) *Reduction of the temperature of storage*

The rate of self-radiolysis is often reduced by cooling the sample to as low a temperature as possible. For solutions, the dispersal of active molecules must remain homogeneous, otherwise a more rapid decomposition may occur than at higher temperatures, due to the formation of local 'pockets' of radiation. This is particularly true for tritium compounds and has been observed for example with frozen aqueous solutions of thymidine-T[40-45] and with solutions of tetrasodium 2-methyl-1,4-naphthaquinol-T diphosphate [27].

LEMMON *et al* [70] showed that the rate of self-radiolysis of the radiation sensitive compound choline chloride-(*methyl*-C14), was reduced by several orders of magnitude by storing the compound at −196 °C (liquid nitrogen). Storage of tritium compounds at this very low temperature was first investigated by APELGOT *et al* [42] for thymidine-T and it has now been shown to reduce greatly the rate of decomposition for many tritium compounds at high specific activity [26].

(c) *Scavenging of the reactive species*

The protective action of benzyl alcohol [26, 40], cysteamine [42], ethyl alcohol [26, 29] and formate [26] against self-radiolysis of tritium compounds stored in aqueous solution has been demonstrated. Ethanol in concentrations of only a few percent, effectively protect amino acids uniformly labelled at high specific activity with carbon-14 [28] (see Table 7), and preliminary results at the Radiochemical Centre indicate that similar amounts of alcohol are effective in reducing the rate of decomposition of solutions of carbohydrates and nucleosides labelled with carbon-14.

Provided the scavenger does not interfere in the tracer experiment, the use of such 'protective agents' promises to be a most useful, practical and convenient method for minimising the decomposition of labelled compounds stored in solution. However, it should not be automatically assumed that the addition of say 1-2% alcohol to an aqueous solution of a radioactive compound will result in a reduced rate of self-radiolysis of the compound. It can be seen from the Table 10 that for some compounds 'scavenging' does not always have any effect; an example is L-methionine-(*methyl*-T).

(d) *Other precautions*

Sometimes the rate of self-radiolysis can be reduced by changing the actual chemical form of the compound. Thus while DL-*nor*adrenaline-(*carbinol*-C14) DL-bitartrate is quite stable, *nor*adrenaline-C14 hydrochloride is not [17]. The difference in radiation sensitivity between choline chloride and choline iodide, is of course another example [64].

Precautions should always be taken against ordinary chemical decompos-

ition. This will include the use of scrupulously clean containers, storage at reduced temperature and in the absence of light, protection against microbiological attack, and, except where protective agents are used, storage as free from contaminants as possible.

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