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Research Article

Radiochemical stability of ^{14}C-compounds on storage: benefits of thioethers

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Summary

Storage of radiochemicals is a significant practical problem. Storage as a solution in various solvents was compared to the storage as a neat oil or solid over an extended period of time. Dichloromethane, a solvent previously not recommended for storage, was found to be a good choice in certain solvent mixtures. Addition of methylsulfide or 2-methyl-2-butene was shown to reduce the radiochemical decomposition by a factor of 1.7–3.2 in ethanol-free solvents. General points to consider for storage of radiochemicals are discussed. Radiochemical purity was determined by HPLC. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: radiochemical purity; storage of radiochemicals; HPLC

Introduction

The development of pharmaceuticals and agrochemicals requires the availability of ^{14}C or tritium labelled compounds which may require storage over an extended period of time. For research purposes the radiochemical purity of the material needed for a study should be at the very least 90%. Regulatory authorities request a higher radiochemical purity for safety studies, i.e. above 95% for active ingredients of

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agrochemicals[1] or >98% for use in humans.[2] Due to internal irradiation, decomposition of labelled compounds occurs much faster than for the non-labelled compound and so frequent re-purification and re-analysis is required.[3] These time-consuming procedures can be reduced by suitable storage. In the present paper, the results of storage as a neat solid or oil, at several temperatures, are compared with those using various solutions at −20°C.

It was observed some time ago that storage in solution may reduce the rate of decomposition of labelled organic compounds.[4] The following criteria should be considered:

(a) The compound has to be soluble at the temperature chosen for storage (−20°C in this study).[5, 7]

(b) Low temperatures are preferred.[4, 5]

(c) The compound under investigation may not be stable in certain solvents such as acetone or alcohols. Among alcohols, ethanol is more reactive than 2-propanol or *t*-butanol.[4, 5]

(d) The pH stability should be taken into account. Acidic or basic degradation products of neutral compounds might alter the pH of a solution.[6]

(e) The protective action of radical scavengers should be utilized. The use of benzylalcohol, ethyl alcohol, formic acid or 2-mercaptoethanol as solvents or solvent components has been reported.[4–6]

Results and discussion

Radiochemical purity was determined by HPLC using a solid-phase radiodetector. For this type of study, it is essential to have accurate results for radiochemical purity. The quantity of material necessary to obtain a precision of 0.2% was investigated. Various concentrations of an aged sample of **6** were injected and the radiochemical purities obtained were compared (Figure 1). At the highest concentration (A) a radiochemical purity of 89.5% was obtained. At a five times lower concentration (B) a radiochemical purity of 90.3% was observed which represents an error of 0.8%, if the former value is correct. At the lowest concentration, detector noise and small impurities are hardly distinguishable giving an apparent radiochemical purity of 93.2% or an error of 3.7%. It is therefore concluded that reliable data for radiochemical purity can be obtained, provided that the signal-to-noise (s/n) ratio

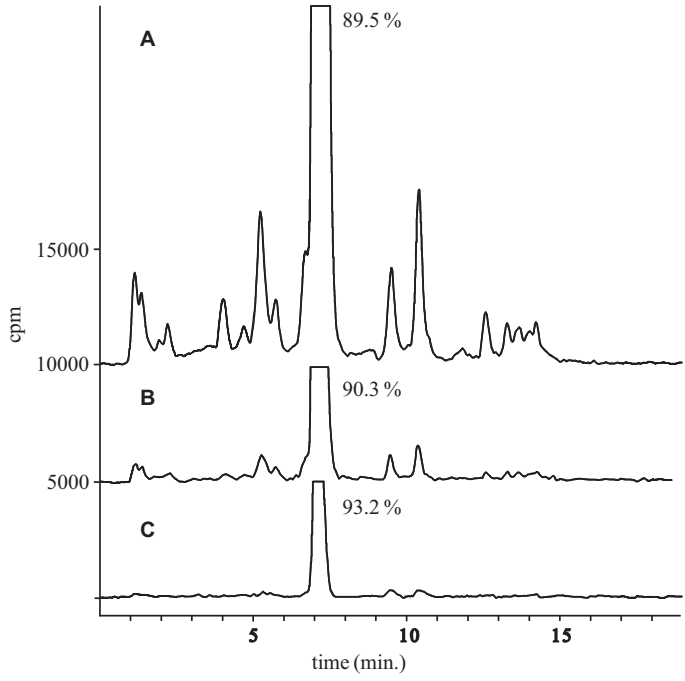

Figure 1. Dependence of radiochemical purity on the radioactivity injected. Approximately 50 kBq of 6 were injected in trace A. A five-fold dilution of that solution gives trace B and a 25-fold dilution trace C. HPLC conditions are given in Table 3

allows the detection of the smallest peaks in the chromatograms representing about 0.1 area %. This is achieved by injecting twice the amount of sample B. It is essential to define the amount injected by the s/n ratio and not by the absolute amount, because only the s/n ratio is independent of the background noise of the solid phase detector cell. No detector-saturation was observed under these conditions, as shown by injecting a small aliquot and counting the collected fractions on a liquid scintillation counter.

The radiochemical stability of ^{14}C labelled compounds varies greatly. Whereas a few compounds decompose with a rate of less than 2% per year if stored as a solid (Figure 3), others deteriorate very quickly. Several compounds (Figure 2) which decomposed rather rapidly were selected for the present study.

In the first series (Table 1) the results of storing various solutions were compared with storage as neat compounds. Oily compounds like **3** or **7** degrade to a purity below 95% within a few months if not stored as

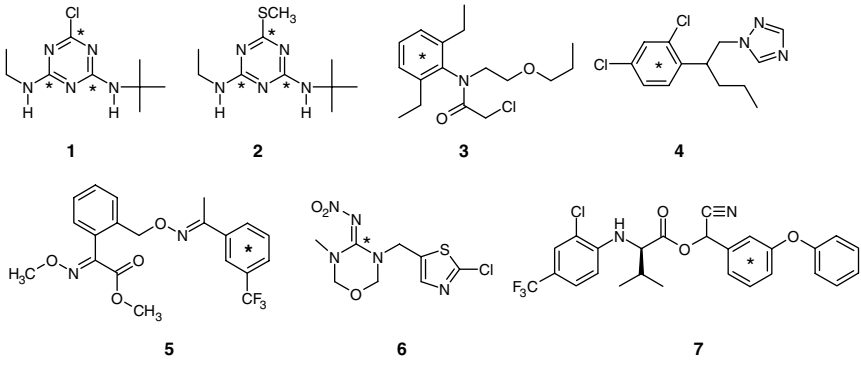

Figure 2. Structures of agrochemicals used in this study: terbuthylazine (1), terbutryn (2), pretilachlor (3), penconazole (4), trifloxystrobin (5), thiamethoxam (6), tau-fluvalinate (7). * denotes the position of the [^{14}C]-label

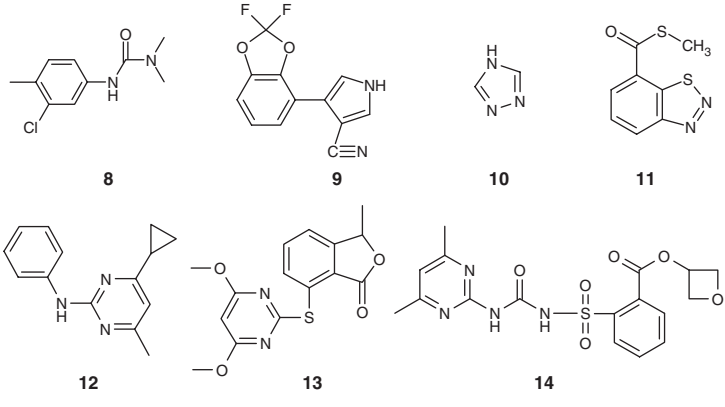

Figure 3. Structures of agrochemicals or heterocycles, which are rather stable if stored as solid (less than 2% decomposition per year at a specific activity of 2 MBq/mg): chlorotoluron (8), fludioxonil (9), 1,2,4-triazole (10), acibenzolar-S-methyl (11), cyprodinil (12), pyriftalid (13), oxasulfuron (14)

solutions. Storage at room temperature or at -70°C influenced the decomposition rate by a factor of less than 2. For all compounds investigated the storage in solution was much more favorable. However the best solvent depended on the compound. Pretilachlor (**3**) is an example of a compound which is soluble in most solvents and where no reactions with solvents are expected. For this type of compound toluene/ethanol-mixtures are usually suitable. Tau-fluvalinate (**7**), however, degrades in the presence of alcohols. Terbuthylazine (**1**) is

Table 1. Radiochemical storage stability of ^{14}C-labelled compounds either neat or in solution

Storage conditions	% Radiolytic decomposition per month						
Compound number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Specific activity (MBq/mg)	7.5	6.9	5.3	3.4	3.7	3.0	2.0
(kBq/mmol)	33.0	29.0	17.0	12.0	9.1	10.3	4.0
Concentration (mg/ml)	2.5	3.5	5.0	5.0	4.0	5.0	6.0
(mmol/l)	11.0	14.0	16.0	18.0	10.0	17.0	12.0
Period observed (months)	12.7	12.6	18.8	39.0	18.9	6.4	25.0
Neat, RT	1.5	1.3	6.4[f]	0.71		1.3	
Neat, −20°C	0.97	1.05	7.9		0.8	0.8	1.4
Neat, −70°C			3.5[f]		0.8	0.8	
In acetone[a]	0.32	0.66	0.36[c]				
In acetonitrile[a]			0.61	**0.17**	0.2	0.56	0.41
In benzene[a]	0.58	0.46					
In benzene/CH_2Cl_2/*methanol* 1 : 1 : 1[a]	0.94[b]	**0.27**					
In dichloromethane[a]						0.94	
In dichloromethane/methanol 1:1[a]	1.2[b]	0.21				**0.063**	
In methanol[a]			0.68	0.23		0.23	
In 2-propanol[a]					**0.14**		
In toluene[a]			0.69	0.27	0.5		0.48
In toluene/ethanol 95:5[a]	0.21	0.24	**0.38**[d]	**0.20**[d]	0.4[e]		

[a] All solutions at −20°C.
[b] Good solvent after 4 months.
[c] Acetone/benzene 95:5.
[d] Toluene/ethanol 90:10.
[e] Toluene/methanol 90:10.
[f] Single measurements.

not very soluble in ethanol (14 mg/ml [8]) or toluene. Compounds **1**, **2** and **6** are most soluble in dichloromethane, which is not recommended in the literature for the storage of radiochemicals.[5] Thiamethoxam (**6**) indeed decomposed rapidly in pure dichloromethane, but – surprisingly – a dichloromethane/methanol mixture is by far the best solvent to store this compound. Also for compounds **1** and **2** similar mixtures were the solvents of choice. It can therefore be concluded, that dichloromethane is a very useful solvent for the storage of otherwise sparingly soluble radiochemicals, if it is used in mixtures with methanol.

The linearity of the radiolytic decomposition in solution was investigated by analyzing 3–4 time points. The decomposition of all compounds was linear in the range observed with the exception of terbuthylazine (**1**). In several experiments the radiochemical decomposition of this compound in dichloromethane-mixtures accelerated considerably after a few percents had been decomposed. Although dichloromethane-mixtures looked very promising after a period of 4

months, unknown degradation products seemed to catalyze further degradation and the speed of breakdown increased. On the other hand, toluene/ethanol 95:5 has the advantage of linear decomposition, but is not suitable for the storage of gram-quantities due to the low solubility of **1** in that solvent.

From the observation that **2** is considerably more stable in solutions of dichloromethane than the closely related compound **1**, it was postulated that sulfur compounds might be beneficial in interrupting radical chain reactions involved in the degradation of radiochemicals. Methylsulfide reacts with hydrogen peroxide forming DMSO.[9] 2-Mercaptoethanol dimerizes under similar conditions but may give unwanted side reactions. In addition 2-methyl-2-butene and the radical scavenger benzyl alcohol were investigated as stability enhancers.

Table 2 summarizes the effect of adding small amounts (1–2%) of stability enhancers. A suitable solvent from the experiment in Table 1 was chosen for this part of the study. In toluene/ethanol mixtures no effect was observed. For all other solvent mixtures investigated, addition of methyl-sulfide increased the stability, most notably in the solvent mixtures containing dichloromethane. 2-Mercaptoethanol was significantly more effective in protecting tau-fluvalinate (**7**) from decomposition, but it is not recommended for storage due to its reactivity with **7**. Addition of 2-methyl-2-butene was beneficial for the storage of the triazines **1** and **2** and to a lesser extent of **7**. Benzyl alcohol has the disadvantage of a high boiling point and is therefore difficult to remove. Furthermore, in no case was it superior to other stability enhancers. In conclusion, an improvement by a factor 1.7–3.2 was achieved in all ethanol-free solvent mixtures by the addition of 2% (v/v) of methylsulfide or 2-methyl-2-butene.

From this study, the following general points may be concluded. It is strongly recommended that all oily compounds be stored as solutions. If the radiolytic decomposition of solids exceeds a rate of 0.5% per month, storage as solutions should be considered. This study should assist in the choice of a suitable solvent. It is noteworthy that addition of methylsulfide is always beneficial, and no case of decreased stability was observed. With solvent mixtures containing ethanol, addition of stability enhancers did not reduce the rate of decomposition. The rate of decomposition is dependent on the concentration. Although storage of larger quantities at very low concentration is favorable for stability reasons, it is impractical due to the handling of large volumes. In our

Table 2. Radiochemical stability of ^{14}C-labelled compounds in solution in the presence of stability enhancers

Storage conditions[a]	% Radiolytic decomposition per month					
Compound number	**1**	**2**	**3**	**4**	**6**	**7**
Solvent used	Benzene/CH_2Cl_2/ MeOH 1:1:1	Benzene/CH_2Cl_2/ MeOH 1:1:1	Toluene/ ethanol 9:1	Toluene/ ethanol 9:1	CH_2Cl_2/ MeOH 1:1	Toluene
Specific activity (MBq/mg)	2.0	2.6	5.3	3.4	2.2	2.0
(kBq/mmol)	8.7	11.0	17.0	12.0	7.5	4.0
Period observed (months)	9.0	27.0	18.8	39.0	26.9	25.0
Concentration (mg/ml)	10.0	16.0	5.0	5.0	15.0	6.0
Without addition	0.41[c]	0.21	0.38	0.20	0.13	0.48
+ water[b]	0.26[c]	0.17[d]			0.15[d]	0.48
+ 2-methyl-2-butene[b]	**0.13**[c]	0.11[d]	0.37	0.18[d]	0.11[d]	0.39
+ methylsulfide[b]	0.18[c]	**0.08**[d]	0.42	0.20[d]	**0.07**[d]	0.42
+ 2-mercapto-ethanol[b]	0.24[c]	**0.08**[d]	0.46	0.20[d]	0.11[d]	0.28[e]
+ benzyl alcohol[b]	0.21[c]	0.16[d]			0.10[d]	
Improvement factor	**3.2**	**2.6**	**0**	**0**	**1.9**	**1.7**

[a] All solutions at −20°C.
[b] 2% (v/v) addition.
[c] Degradation is accelerated 2–3 fold after a period of 27 months.
[d] 1% (v/v) addition.
[e] Not suitable: material decomposes upon evaporation of solvent.

Table 3. HPLC methods

Compound	Column	Eluent	Gradient		Detection (nm)	*Rt* (min.)	Sample dissolved in
1	[a]	A: H_2O/TFA 100:0.1	0 min	35% B	220	7.3	Methanol
		B: acetonitrile/TFA 100:0.075	10 min	45% B			
			15–17 min	100% B			
2	[a]	A: H_2O/TFA 100:0.1	0 min	25% B	225	7.0	Methanol
		B: acetonitrile/TFA 100:0.075	10 min	35% B			
			15–17 min	100% B			
3	[a]	A: H_2O/TFA 100:0.1	0 min	50% B	218	9.1	Acetonitrile
		B: acetonitrile/TFA 100:0.075	10 min	60% B			
			14–17 min	100% B			
4	[a]	A: H_2O/TFA 100:0.1	0 min	45% B	220	6.3	Methanol
		B: acetonitrile/TFA 100:0.075	10 min	55% B			
			15–17 min	100% B			
5	[a]	A: H_2O/H_3PO_4 100:0.1	0 min	55% B	254	7.1	Acetonitrile
		B: acetonitrile	10 min	65% B			
			14–17 min	100% B			
6	[a]	A: H_2O/TFA 100:0.1	0 min	8% B	252	6.5	Methanol
		B: acetonitrile/TFA 100:0.075	10 min	18% B			
			15–17 min	100% B			
7	[b]	A: H_2O/TFA 100:0.1	0 min	60% B	218	7.0	Acetonitrile
		B: acetonitrile/TFA 100:0.075	10 min	65% B			
			12–15 min	100% B			

[a] Nucleosil 100-5 C18, 125 × 4.0 mm.
[b] LiChrospher RP select B, 5 μm, 125 × 4.0 mm.

experience concentrations in the range of 5–20 mg/ml are suitable for storage of batches with a specific activity of 2 MBq/mg.

In summary, it has been shown that storage of radiochemicals in solution is frequently favorable. Addition of ethanol, 2-methyl-2-butene, or methylsulfide enhances the storage stability considerably. From the fact that thioethers reduce radiolytic decomposition, it can be speculated that sulfur-rich food like garlic is helpful to treat humans from radiosickness. Maybe garlic is not only helpful against *B. Stoker*'s Dracula, the vampire of the 19th century, but also against the nightmare of the atomic age.

Experimental

Chemicals: All ^{14}C-labelled materials were purified or prepared in our laboratory. Water for chromatography was from Novartis Services. Acetonitrile (isocratic grade for LC), TFA (purum), ortho phosphoric acid (puriss.), 2-propanol (for HPLC), 2-methyl-2-butene (puriss.), methylsulfide (puriss.) and benzyl alcohol (puriss.) were from Fluka, Buchs, Switzerland. 2-Mercapto-ethanol was from Sigma (St. Louis, MO, USA). The following solvents were from Merck (Darmstadt, Germany; all p.a.): acetone, benzene, dichloromethane, toluene, methanol (gradient grade).

HPLC: The liquid chromatograph setup consisted of two Shimadzu LC-10AD pumps, an autoinjector SIL-10A, an SPD-M10A diode array detector (Shimadzu, Kyoto, Japan) and a Berthold LB 506 B (EG & G Berthold, Bad Wildbad, Germany) equipped with a YG solid scintillation cell of 150 µl. Parameters: Nuclide 14C; Cell Model YG; Ratem. Units: min.; H-Backgnd: 0 cpm; Eff. Correct.: no; H-Range 500 K cpm; Peak-FWHM: 8 s; H-time C 1.5 FWHM. Integration was achieved by A/D converter CMB-10A and LC-10 software version 1.6 (Shimadzu). The HPLC methods used are summarized in Table 3. Columns are cartridges from Macherey–Nagel (Düren, Germany).

Storage solutions: In a typical experiment 0.7 mg of material was transferred into a 1.5 ml flask and dissolved in 200 µl of the appropriate solvent mixture. For analysis 40 µl was taken from that solution, the solvent removed by a stream of nitrogen and dissolved in 200 µl of a suitable solvent (Table 3). Larger quantities were stored in crimp seal vials sealed with a Teflon-coated septum.

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