CARRIER-FREE ^{11}C FORMALDEHYDE : AN APPROACH.

G. Berger, M. Mazière, J. Sastre and D. Comar.

Commissariat à l'Energie Atomique, Département de Biologie,

Service Hospitalier Frédéric Joliot,

91406 - ORSAY, France.

SUMMARY

The different parameters of ^{11}C formaldehyde synthesis from ^{11}CO$_2$ have been systematically studied in order to improve yields, reproductibility and specific radioactivity.

With modifications brought to gas and reagents purity, ^{11}CO$_2$ trapping system, target and dehydrogenation catalyst treatment, 100 to 200 mCi of ^{11}C formaldehyde are obtained at EOB + 10 mn, with a specific activity on the order of 600 mCi/μmole (maximum 1 Ci/μmole).

Key Words : Carbon 11-formaldehyde carrier-free.

INTRODUCTION

One of the main advantage of using ^{11}C as a label for organic molecules is the very high specific activity theoretically attainable. Under such conditions, trace amounts of biological compounds of high molecular weight or of drugs may be injected in man for diagnostic purposes under physiological or non-toxic conditions. In spite of the numerous publications describing ^{11}C labelling of organic compounds **nobody** has yet obtained specific activities anywhere near the theoretical value of 10^4 Ci/μmole. The best results are those of Christman and coll. (1) with H^{11}CN : 1 to 2 Ci/μmole. In previous publications, methods for the synthesis of ^{11}C formaldehyde (2, 3) and ^{11}C methyliodide (3) as precursors for a number of mole-

cules of medical interest were described. In spite of the care taken
to avoid sources of contamination by stable carbon, only low speci-
fic activities could be obtained (15-30 mCi/μmole). In the present
work all the parameters involved in the synthesis of ^{11}C formaldehy-
de were studied and discussed in order to apprehend the origin of
these contaminations and propose new solutions to the problem. The
different steps of the synthesis are the production of $^{11}CO_2$, its
transformation into ^{11}C methanol, which by catalytic dehydrogena-
tion leads to ^{11}C formaldehyde.

MATERIAL AND METHODS.

1) Preparation of $^{11}CO_2$.

$^{11}CO_2$ is obtained from the nuclear reaction $^{14}N(p,\alpha)^{11}C$ by
bombarding nitrogen (Air Liquide N 60) with 20 MeV protons, traces
of oxygen (< 0.1 ppm) present in the gas sufficing to produce $^{11}CO_2$.

The target (volume = 650 ml, length = 40 cm) containing the
gas under pressure (7 bars) is made of AG3 aluminium and the inlet
foil (thickness 0.3 mm) of pure aluminium. Cooling is obtained by a
water flow in double walls and by helium in front of the target.
All organic joints have been avoided.

After irradiation the pressure in the target is released and
the radioactive carbon dioxide is carried by the current of nitrogen
into stainless steel or rilsan tubes and trapped in a copper coil
cooled to -183°C (liquid oxygen). 6.5 litres of nitrogen pass through
the trap during this operation.

2) Preparation of ^{11}C methanol.

The apparatus is the same as described earlier (3) (Fig. 1).
The $^{11}CO_2$ is released by warming the coil (20°C), carried by a
nitrogen-oxygen mixture (Air Liquide nitrogen N 48 containing 2%

oxygen N 45, flow-rate 100 ml/mn) and collected in a three-necked

flask containing about 15 μmoles lithium aluminium hydride $\left(\text{LiAlH}_4\right)$

dissolved in 100 μl tetrahydrofuran (THF) at -20°C. The THF is evapo-

rated by heating $\left(t = 70°C, 1 \text{ to } 2 \text{ mn}\right)$, the flask is cooled again

to -20°C and the compound formed $\left(\text{AlLi}\left(\text{OCH}_3\right)_4\right)$ is decomposed by

0.2 ml water. The ^{11}C methanol produced is carried off by the gas

mixture (flask heated to 100°C, flow-rate 100 ml/mn) through a

Porapak P trap (length 3 cm, diameter 0.7 cm), collected in 0.5 ml

water cooled to 4°C and analysed by gas chromatography (Varian,

series 1400, Porapak P column : length = 2 m, diameter = 1/8 inch,

column temperature = 90°C, injection and detection temperature =
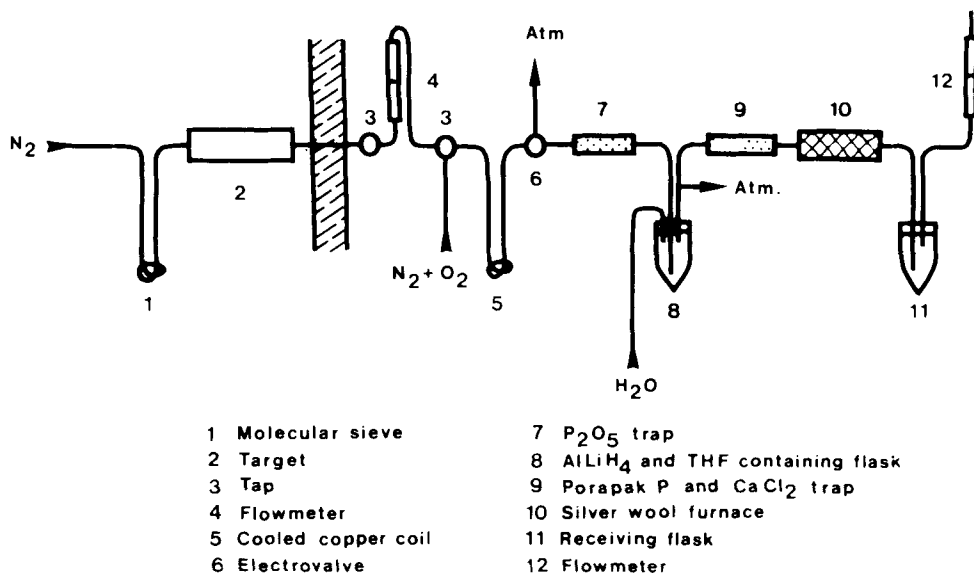
200°C, N_2 vector gas, flow-rate = 30 ml/mn).

1 Molecular sieve	7 P_2O_5 trap
2 Target	8 AlLiH$_4$ and THF containing flask
3 Tap	9 Porapak P and CaCl$_2$ trap
4 Flowmeter	10 Silver wool furnace
5 Cooled copper coil	11 Receiving flask
6 Electrovalve	12 Flowmeter

Fig. 1 - Apparatus for production of ^{11}C formaldehyde.

3) Preparation of ^{11}C formaldehyde.

The gas mixture leaving the Porapak P trap passes into a fur-
nace (length = 12 cm, diameter = 1 cm) containing 2 g silver wool
(Prolabo) distributed over about 7 cm and heat-regulated. The opti-
mum temperature is determined periodically by tests with non-radio-
active methanol. Before each manipulation involving ^{11}C the catalyst
is "activated" by passing through 0.2 mg methanol in 0.2 ml water at
the optimum temperature.

The ^{11}C formaldehyde is collected in 0.5 ml water at $4°C$ or in
0.5 ml dimethylformamide cooled to $-20°C$. It is measured by T. Nash's
method (4) and its radiochemical purity determined in various ways :
- selective precipitation with dimedone : to 400 µl formaldehyde M
carrier are added 10 µl of the solution to be analysed and 10 ml
dimedone solution (1.6 g dimedone, 30 ml methanol, made up to 100 ml
with water). The mixture is heated for 10 mn at 100°C, cooled and
filtered ;
- gas chromatography : same conditions as for methanol analysis ;
- high pressure liquid chromatography : elution is carried out on a
column of µ Porasil (Waters Associates) by a mixture of chloroform
(97%) and ethanol (itself containing 2.5% water and 1.5% ethylamine).

RESULTS

1) A 30 minute 20 µA irradiation with 20 MeV protons yields between
400 and 600 mCi ^{11}CO$_2$ recuperated in the trap.

It should be noted that the activity cannot all be collected by
simple decompression of the gas from 7 bars to atmospheric pressure.
This could only be achieved by further sweeping but at the cost of
added carrier gas.

2) The reduction of carbon dioxide to methanol is satisfactory : if known quantities of stable CO_2 (0.5 to 3 µmoles) are injected, the yield obtained with this apparatus, after 5 mn sweeping at a rate of 20 ml/mn and collection of the methanol in two traps in series, is 87% (curve 2). The simple methanol transfer takes place with 90% efficiency.

In manipulations with ^{11}C with no added carrier, 70 to 90% of the activity is incorporated in the methanol. 200 to 400 mCi are recuperated (EOB + 10 mn).
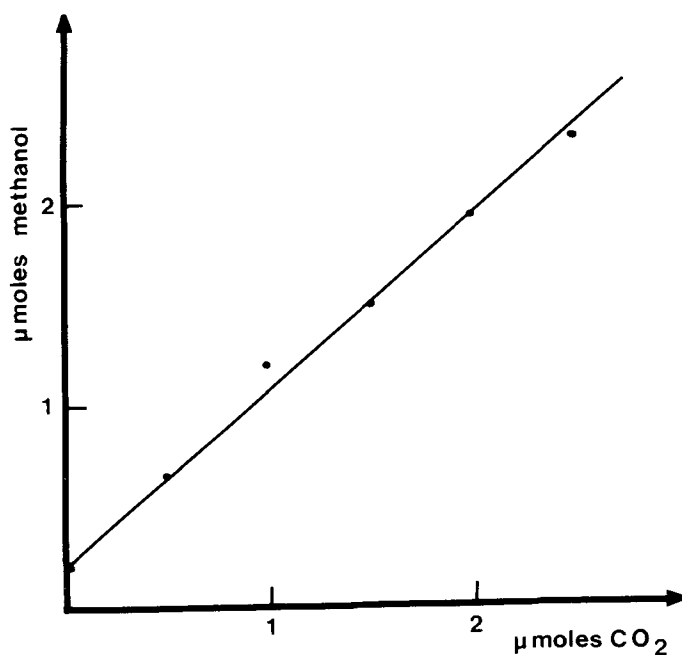

Fig. 2 - Reduction of CO_2 into methanol.

- see text -

3) The methanol dehydrogenation stage on the other hand is more cri-
tical : the optimum working temperature of the furnace must be deter-
mined beforehand. With a furnace containing 2 g silver, swept for
5 minutes by a 100 ml/mn current of vector gas, the maximum yield
from methanol lies between 50 and 75% according to the state of acti-
vity of the catalyst (curve 3). 80 to 200 mCi of ^{11}C formaldehyde
are obtained (EOB + 10 mn). The quantities are in general smaller
than could be expected, considering the yields of the various opera-
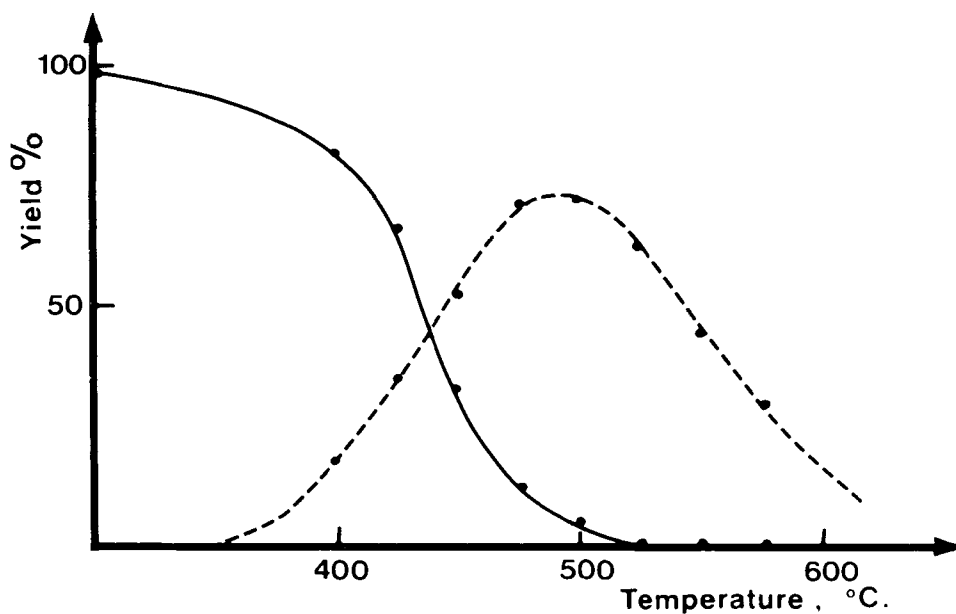tions measured under non-active conditions.

Fig. 3 - Dehydrogenation of methanol into formaldehyde
with silver catalyst.
—— methanol
---- formaldehyde

4) Specific radioactivity : the ^{11}C methanol and ^{11}C formaldehyde specific radioactivities approximate 600 mCi/μmole (average on 25 experiments) when these precursors are ready, i.e. at EOB + 10 mn. The maximum obtained is 1 Ci/μmole.

Although the method has been considerably improved since it was last described (3), the stable carbon dilution is still substantial.

DISCUSSION

1) [11]CO_2.

The amount of [11]CO_2 produced depends on the nitrogen pressure (7 bars under 10 μA irradiation corresponds to 6 bars in the absence of ionisation produced by proton bombardment) and target length and is proportional to the proton beam intensity. However the quantity of radioactivity released from the target is variable and a large amount is held back after a certain number of operations. Some of the activity probably stays fixed on the alumina coating of the target surface (5) since preliminary washing of this latter with HCl or H_3PO_4 N/50, or injections of dilute HCl, greatly improves the [11]CO_2 recuperation.

2) ^{11}C methanol.

A small fraction of the activity (about 10%) is left behind after hydrolysis in the three-necked flask containing lithium aluminium hydride : when the $LiAlH_4$ concentration is insufficient or the temperature too low the carbon dioxide may not be completely reduced (formic acid stage), but too great an excess of hydride would lower the specific activity. Another methanol fraction (about 10%) is not trapped in the receiving tube but is recovered if a second tube is placed in series with the first.

In any case the transformation is satisfactory (70 to 90%).

3) ^{11}C formaldehyde.

The quantities of ^{11}C formaldehyde are generally smaller than would be expected from the yields of the different stages measured with inactive products.

This was partly due to the presence of trace amounts of THF deactivating the catalyst. Lithium aluminium hydride fixes ethers (6) in the form of complexes which only dissociate under high vacuum. These traces are released by hydrolysis and come into contact with the silver, causing a sharp drop in the formaldehyde/methanol ratio at a given temperature. Furthermore in the presence of oxygen and silver, THF decomposes to form methanol, formaldehyde and acetaldehyde. The specific activity therefore falls and the acetaldehyde can compete with formaldehyde in subsequent labellings.

A Porapak P trap at the flask outlet holds back THF sufficiently with respect to methanol and so avoids these disadvantages. It is generally followed by another trap of calcium chloride to stop hydrolysis water, which inhibits certain methylation reactions (7). Certain poor yields are due to changes in the surface state of the silver during heating. A reduction in the maximum of curve 2 and a displacement towards low temperatures are observed. In manipulations involving ^{11}C the furnace is often heated and "activated" an hour before the passage of ^{11}C methanol, for practical reasons. The displacement is difficult to estimate. Moreover the calibrations are carried out with several µmoles of stable methanol whereas in active work these quantities are smaller (0.2 to 0.5 µmole), and adsorption in the different parts of the apparatus (traps, tubes, silver) is probably greater. It should be noted finally that the temperatures given for the furnace are somewhat arbitrary : the thermocouple is situated outside the quartz tube containing the silver and because

of the gas flow-rate a temperature gradient exists. The thermocouple must therefore be well fixed and the flow-rate reproducible.

4) Specific radioactivity.

Different factors were examined one after another with a view to improving the specific activity.

- The $^{11}CO_2$ was formerly recuperated on molecular sieve (Merck, 5 Å, 60-80 mesh). However this substance when heated gives off small quantities of carbon dioxide (1 μmole CO_2 in 15 mn at 200°C). It is possible to elute the $^{11}CO_2$ at lower temperature by reversing the direction of the gas flow and miniaturizing the trap, but this source of pollution can also be completely eliminated by trapping in a metal coil cooled to -183°C with liquid oxygen.

- For the hydrolysis it is preferable to use water freshly distilled and decarbonated by boiling.

- In the course of its manufacture, during its dissolution in THF and when it is sampled lithium aluminium hydride absorbs atmospheric carbon dioxide : between 0.01 and 0.1 μmole CO_2 (average : 0.04 μmole) for the 15 μmoles of product added.

- The nitrogen used contains traces of CO_2 : the contribution from N 48 (Air Liquide) is estimated at about 0.1 μmole (for 6.5 litres of gas) and that from N 60 at 0.06 μmole. Moreover under irradiation any impurity liable to radiolyse into CO_2 (such as CH_4) provides an additional source of isotopic dilution. A trap made of 5 Å molecular sieve cooled to -20°C and placed before the target improves the results, especially in the case of nitrogen N 48.

- The possibility of some radiolysis of the rilsan pipes by cyclotron-scattered radiation has been ruled out : the doses received are of the order of a Krad (measured by Fricke dosimetry) which is not enough to produce an appreciable reaction. The secondary effect of

[11]C activity on the tubing of the apparatus, on the THF and on the chromatographic detector flame ionisation is negligible since the amounts of carrier found when the [11]CO_2 is left to decay beforehand in the target are the same.

- Permeability of the target inlet foil to atmospheric carbon dioxide seems to be out of the question : injection of CO_2 in front of this foil, the fact that this is made of titanium or aluminium, hardly affect the results.

- An important source of carrier is to be found within the target under irradiation : proton bombardment of helium leads to the production of stable CO_2 in quantities comparable to those obtained with nitrogen.

This source was identified as the organic joint (nitrile PD 501, le Joint Français) used formerly to seal the inlet foil. Its replacement by a less radiation-sensitive substance (ethylene-propylene EP 851, le Joint Français), then by a joint of aluminium sunk in indium, increased the specific activity by a factor 5. Since little radiation is scattered outside the beam it is likely that destruction of the polymer is due to a phenomenon similar to that of chemical ionisation used in mass spectrometry, nitrogen radicals being the ionising agents whereby the carbon-containing fragments produced are then radiolysed to CO_2.

Whatever kind of target is used (with or without organic joint, walls of quartz, aluminium AG3 or gold-and platinum-plated) the specific activity rises during irradiation (figure 4) if the CO_2 produced is swept out continuously. Since a constant supply of carbon is added (organic joint or atmospheric leakage) an equilibrium is set up, dependent on the rate at which the two phenomena occur. At present it seems that the faulty tightness of the circuit is responsible for the carbon input (the partial pressure of atmospheric

CO_2 is more than 1000 times higher than that in the gas), although
slow diffusion of deep carbon present in the AG3 alloy (0.05 to
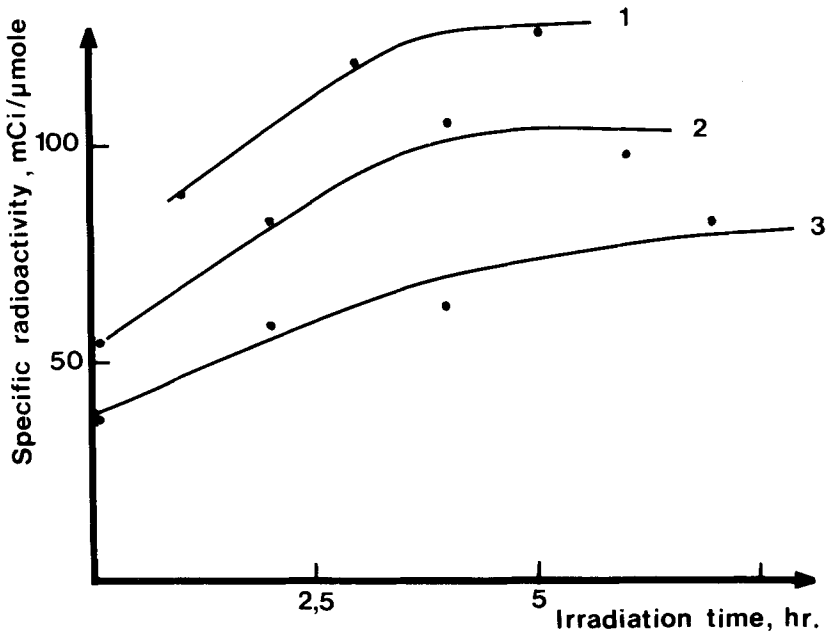0.25%) may also be suspected.

Fig. 4 – Specific radioactivity versus irradiation time
under different conditions.
Irradiations by 20 MeV protons, 15 μA.
The inlet foil is sealed with an organic joint.
1) Titanium inlet foil, gas N 60, metallic pipes.
2) Titanium inlet foil, gas N 48.
3) Four titanium foils, gas N 48 purified on molecular sieve.

CONCLUSION

Owing to the various modifications described it is now possible to obtain ^{11}C formaldehyde in more regular quantities and to improve its specific radioactivity by a factor of about 25. Molecules can thus be ^{11}C formaldehyde labelled (7, 8, 9) with a specific activity when available (after about 40 minutes) between 100 and 300 mCi/μmole : 10 mCi of these substances represent 0.1 to 0.03 μmole, which is below the physiological tolerance threshold for many poisonous materials.

Further progress may be made by eliminating all external sources of carbon, by purification of the gas N 60 on molecular sieve, avoiding pollution of the lithium aluminium hydride, and using the maximum proton beam intensity. It is hoped in this way to gain a factor 2.

It is obvious that with this method it will become more and more difficult to progress as the amounts of CO_2 involved are smaller, until the detection limits are reached.

ACKNOWLEDGMENTS

The authors are grateful to Mr R. Knipper for the realization the gas-tight target with metallic joint.

BIBLIOGRAPHY

1. D.R. Christman, R.D. Finn, K.I. Karlstrom, A.P. Wolf,
   Int. J. Appl. Rad. Isotopes, 26:435-442 (1975).

2. D.R. Christman, F.J. Crawford, M. Friedkin, A.P. Wolf,
   Proc. Nat. Acad. Sci. USA, 69:988-992 (1972).

3. C. Marazano, M. Mazière, G. Berger, D. Comar, Int. J. Appl. Rad.
   Isotopes, 28:49-52 (1977).

4. T. Nash, Biochem. J. <u>55</u>:416-421 (1953).

5. P. Pascal, in "Nouveau traité de Chimie minérale", <u>6</u>:557-573 (1961)
   Masson et Cie editors, Paris.

6. W.G. Brown, Organic reactions, <u>6</u>:469-509 (1951).

7. M. Mazière, G. Berger, D. Comar, J. Radioanalytical Chem.
   (in press).

8. M. Mazière, J. Sainte-Laudy, M. Crouzel, D. Comar, Radiopharma-
   ceuticals, G. Subramanian editor, New York, Society of Nuclear
   Medicine 189-195 (1975).

9. M. Mazière, D. Comar, C. Marazano, G. Berger, Eur. J. Nucl. Med.
   <u>1</u>:255-258 (1976).