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Automated Synthesis of ^{11}C-Labelled Radiopharmaceuticals: Imipramine, Chlorpromazine, Nicotine and Methionine

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This article describes a method by which about a hundred mCi of different radiopharmaceuticals (imipramine, chlorpromazine, nicotine or methionine) may be prepared in 30–35 min with no irradiation risk to the operators. All operations (preparation of precursor, methylation of the monodesmethyl derivative, purification and sterilisation of the end product) are carried out in a closed shielded hood. The passage of fluids from one reaction tube to another through flexible teflon capillaries is controlled by electrovalves and purification is achieved by HPLC. The end products are chromatographically pure, sterile and apyrogenic. Their specific radioactivity at the time of use averages 500 mCi/μmol (maximum 1.0 Ci/μmol).

Introduction

DURING the last few years the use of cyclotron-produced short-lived radioelements has become more and more popular, but in most cases it involves a manual intervention to incorporate the radioelement obtained into an organic molecule.

The short half-life of ^{11}C (20.4 min), a definite advantage in medical use because of the low irradiation dose to the patient, is troublesome for the organic chemist who has a preparation to make for each diagnostic test and must work with strong radioactivities in order to arrive at a high enough injectable activity. Because of the radiation emitted (β^+, E_{max} 0.97 MeV), ^{11}C produces a dose rate of 15 Rem/h/mCi through a 2 mm thick glass container and therefore cannot be handled without adequate shielding.

Moreover these new radiopharmaceuticals are unsuitable for routine or medical use unless the quality, sterility and apyrogenicity of the labelled molecule can be guaranteed.

For these reasons we have developed an automatic synthesis method where all operations take place in a closed shielded hood and ^{11}C can be incorporated without manual intervention.

The molecules involved in this study are imipramine, chlorpromazine, nicotine and methionine.

Principle of the Method

The process takes place in three stages:

(1) *Preparation of a precursor*: $I^{11}CH_3$ or $H^{11}CHO$. ^{11}C is formed by $^{14}N(p, \alpha)\ ^{11}C$ reaction and combines immediately with traces of oxygen in the target to give $^{11}CO_2$. This is reduced by aluminium lithium hydride ($AlLiH_4$) in tetrahydrofuran (THF) solution. The $Al\text{–}Li(O^{11}CH_3)_4$ complex formed is hydrolysed and gives off labelled methanol.

According to requirements the methanol is either dehydrogenated into formaldehyde by passage over silver at 500°C in the presence of oxygen or converted into methyl iodide by bubbling through hydriodic acid under reflux. These methods have already been described.[1–3]

$$^{14}N \xrightarrow{(p\alpha)} {}^{11}C \xrightarrow[\text{traces } O_2]{} {}^{11}CO_2$$

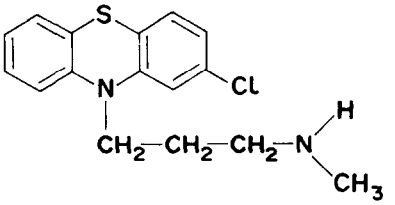
$$4{}^{11}CO_2 + 3AlLiH_4 \longrightarrow AlLi(O^{11}CH_3)_4 + 2AlLiO_2$$

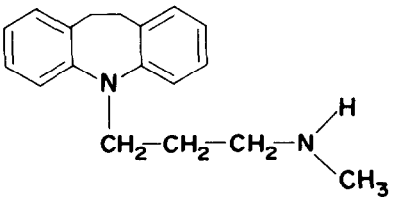
$$AlLi(O^{11}CH_3)_4 + 4H_2O \longrightarrow Al(OH)_3 + LiOH + 4{}^{11}CH_3OH$$

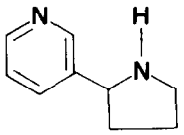
$$^{11}CH_3OH \xrightarrow[\text{IH on reflux}]{} {}^{11}CH_3I + H_2O$$

$$^{11}CH_3OH \xrightarrow[\text{Ag. 500 . } O_2]{} H^{11}CHO + H_2O$$

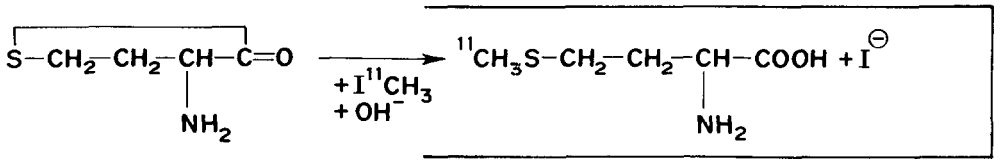
(2) *Methylation.* This is obtained by reaction of formaldehyde with monodesmethyl derivatives of chlorpromazine, imipramine or nicotine. The imine formed is reduced by sodium cyanoborohydride according to BORCH's method:[4–6]







In the case of methionine, methyl iodide reacts with L-homocystein thiolactone in basic solution in the presence of acetone:[7]



(3) *Purification.* The labelled products are purified by HPLC, on a silica column for imipramine, chlorpromazine or nicotine eluted with chloroform ethanol mixtures, on grafted silica for methionine using reverse phase elution with physiological serum buffered to pH 3.3. Sterilisation is obtained by passage over 0.22 μMillipore filter, after evaporation of the solvent and redissolution of the product in buffered physiological serum if necessary.

Materials and Methods

Materials

The reaction tubes are cylindroconical (1 ml to 2.5 ml), plugged by chromatographic septa (Carlo Erba), interconnected by teflon capillaries (θ int. = 0.08 cm) fixed onto medical needles. Passage of the fluids is governed by Durrum electrovalves remotely controlled by compressed air.

The containers are manipulated through rods and can be transferred from a cold bath (ice–ethanol: $T° = -10$ to -15°C) to a hot bath (water or Wood's alloy, $T° = 50$, 70 or 130°C as the case may be).

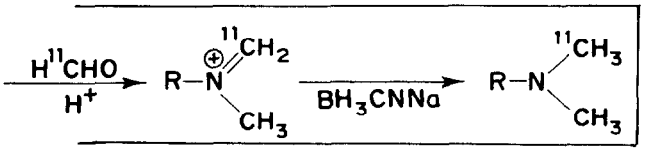
The chromatograph (Waters Ass.) consists of a 6000 A pump, a U6K injector and a M440 absorbancy detector or a R401 refractometer. The columns (Whatman) are Partisil 1050 or 10 ODS 50 Magnum 9.

The radioactivity transfers are followed by three LCRI ionisation chambers: one at the cooled metal tube where the $^{11}CO_2$ is collected, another beside the tube in which the methylation reaction takes place and the third at the chromatograph outlet (see Fig. 1).

All this equipment is housed in a hood shielded by 5 cm of lead (Fig. 2).

Operational sequence

Nitrogen under 7 b pressure, irradiated for 30 min with 20 MeV protons at intensity 25 μA, is released by simple decompression and the $^{11}CO_2$ formed is carried by the nitrogen current through a P_2O_5 trap ($l = 6$ cm, $\theta = 0.3$ cm), where traces of water are held back, and trapped in a metal capillary ($l = 40$ cm, $\theta_{int} = 0.1$ cm) immersed in liquid oxygen (−183°C).



This operation takes about 5 min and an activity between 500 mCi and 1 Ci is recuperated according to the state of the target wall, the alumina of which fixes part of the $^{11}CO_2$.[8]

The trap is warmed to 20°C to release the activity, which is then carried by a gas current (nitrogen N 48 + 2% oxygen N 45, flow rate 20 ml/min) into the first tube containing 50 μl anhydrous THF and 4 to 8 μmol $AlLiH_4$ (2.5 to 5 μl of ~ 1.5 M solution), added beforehand and kept at −10 to −15°C. This transfer takes 10–20 s. The passage of the gas is controlled by electrovalve I, operated by the first remote control module.

The THF is evaporated by heating the tube for a few seconds in a furnace at 130°C, then, after cooling, 50 to 100 μl of water are introduced with a syringe. The hydrogen formed by decomposition of excess hydride escapes to the outside.

By action of the 2nd and 3rd remote control modules on electrovalves II, IV, III and V, communication is established with the tube containing the reaction mixture kept at −10°, −15°. The reaction tube is again heated to 130°C and the methanol distils off.

In the case of ^{11}C-formaldehyde preparation the methanol is carried through a Porapak P trap ($l = 10$ cm, $\theta = 0.3$ cm) to stop traces of THF, then through a quartz tube containing 0.5 g silver wool ($l = 7$ cm, $\theta = 0.3$ cm), at a previously calibrated temperature (about 500°C) where it is dehydrogenated. The silver catalyst is changed each time.

For the preparation of ^{11}C-methyl iodide the ^{11}C-methanol is bubbled in a tube ($V = 2.5$ ml) containing 200 μl of 67% hydriodic acid under reflux, then passes through a trap ($l = 12$ cm, $\theta = 0.3$ cm) containing soda lime granules ($l = 9$ cm) and phosphoric anhydride ($l = 1$ cm) to hold back the hydriodic acid and water present.

The arrival of the radioactivity in the methylation tube is followed by means of an ionisation chamber.

The reaction mixture for imipramine, chlorpromazine or nicotine synthesis consists of 1 μmol monodesmethyl derivative, 1 μmol sodium cyanoborohydride, 2 μl acetic acid, 50 μl water and acetonitrile qsp 250 μl.

For the preparation of methionine the $I^{11}CH_3$ is trapped in 200 μl acetone and the homocystein thiolactone solution (2 μM in 150 μl water, 70 μmol NaOH) is then added from outside with a syringe.

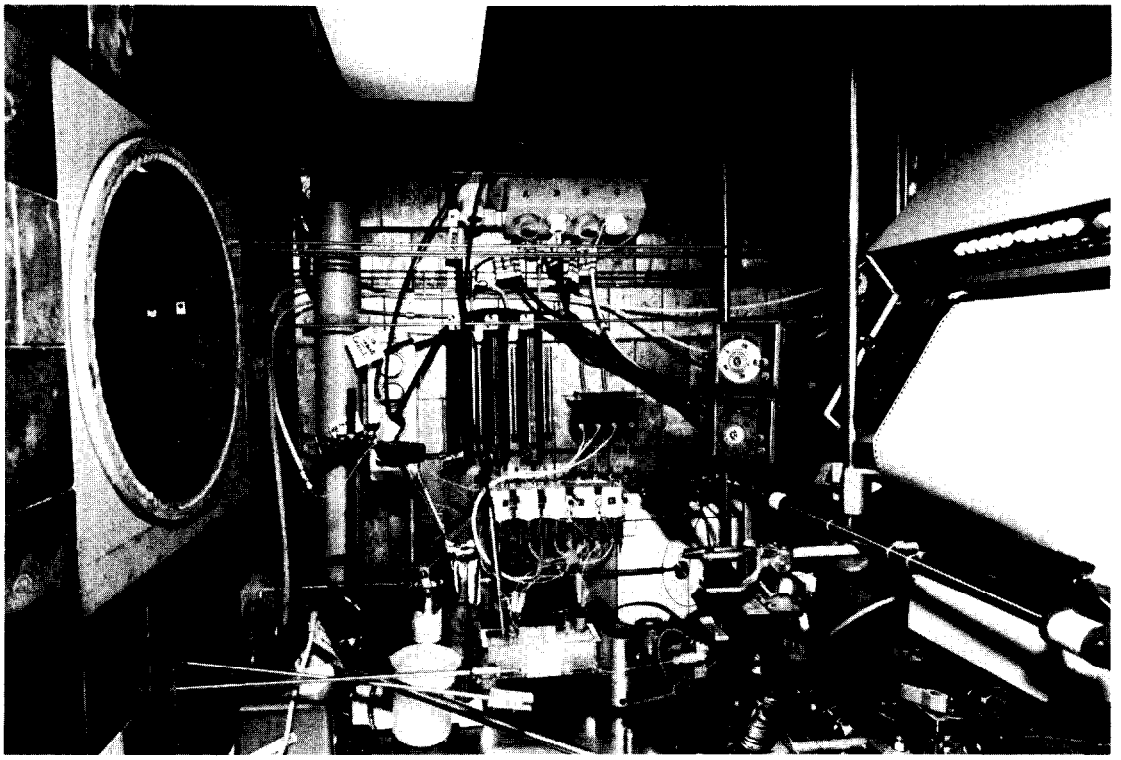


FIG. 2. Inner view of the lead shielded cell where ^{11}C-chlorpromazine, imipramine and nicotine are prepared.

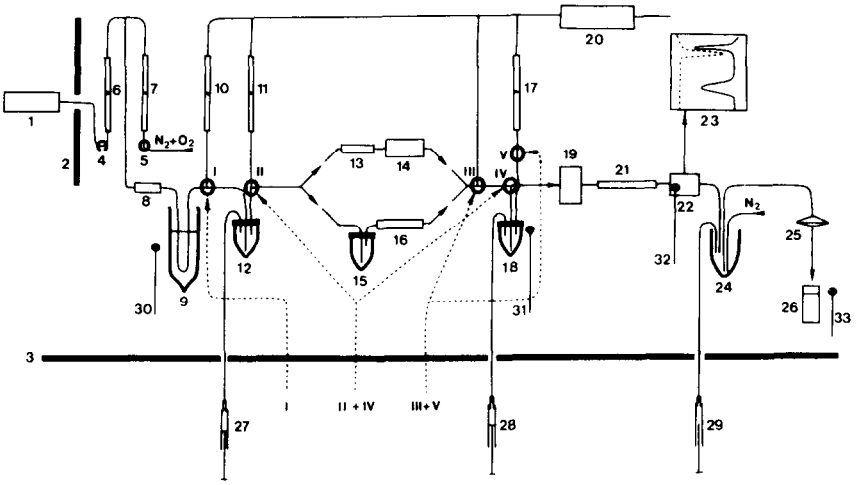


FIG. 1. Diagram of the apparatus; (1) target, (2) concrete wall, (3) 5 cm thick lead wall, (4, 5) taps, (6, 7) flowmeters, (8) P_2O_5 trap, (9) metal capillary, cooled with liquid oxygen, (10, 11) flowmeters, (12) reaction tube, (13) Porapak P trap, (14) silver furnace, (15) reaction tube, (16) soda lime and P_2O_5 trap, (17) flowmeter, (18) reaction tube, (19) U 6 K injector, (20) soda lime filter, (21) chromatography column, (22) u.v. detector or refractometer, (23) chromatogram recording, (24) receiving tube, (25) millipore filter, (26) sterile container, (27) syringe containing water, (28) syringe for homocystein or air injection, (29) syringe containing buffered physiological serum, (30, 31, 32, 33) ionisation chambers, (I, II, III, IV, V) electrovalves.

When all the radioactivity ($H^{11}CHO$ or $I^{11}CH_3$) has been transferred (3–5 min) the tube is isolated by action of the remote control module on electrovalves III and V and its temperature brought to 50°C for 7 min in the case of chlorpromazine and imipramine, or 10 min for nicotine and to 70°C for 7 min in the case of methionine.

The reaction mixture is then injected into the chromatograph by the pressure of 1–1.5 ml air introduced with a syringe, electrovalve III being in the right position. The injector valves are locked from outside.

Chlorpromazine and imipramine are eluted on a 50 cm Partisil Magnum 9 column by a mixture of 97% chloroform, 3% ethanol (itself containing 1.5% ethylamine and 2.5% water). The nicotine elution mixture is similar (95/5 v/v). The flow rate is 8 ml/min and the optical density measured by a M440 spectrometer at 254 mμ. The fraction corresponding to the appropriate chromatogram peak is collected in a heated container (water-bath: $T° \simeq 70°C$) and the solvent (5–10 ml) evaporated by nitrogen bubbling, a few drops of hydrochloric ether having been added in the case of nicotine. The labelled product is redissolved in 5 ml physiological serum buffered to pH 3.3 by sodium phosphate ($2.5.10^{-3}$ M).

Methionine is eluted on a 50 cm Partisil ODS 2 Magnum 9 column by the buffered physiological serum described above (flow rate 6 ml/min) and the refraction index of the eluate is followed with a R401 refractometer.

The solutions are sterilised by passage over 0.22 μm Millipore filter connected by a sterile needle to an evacuated sterile flash, the pressure difference being enough to operate the transfer.

The syringe intended for the injection may also be filled directly: an electrovalve is placed before the Millipore filter and the sterile syringe is kept in the drawn-out position

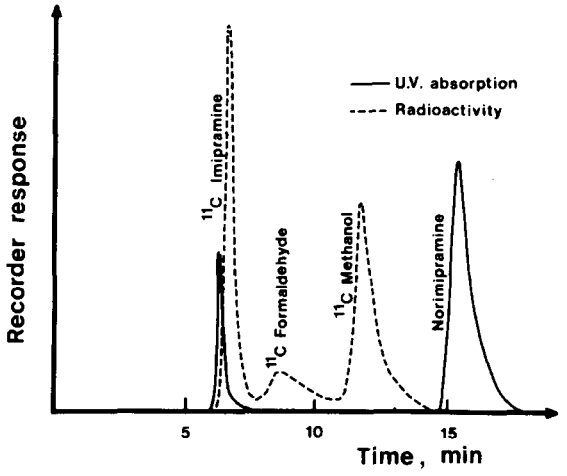


FIG. 3. Chromatograph of a synthetic mixture where reaction has not been total. Conditions described in the text.

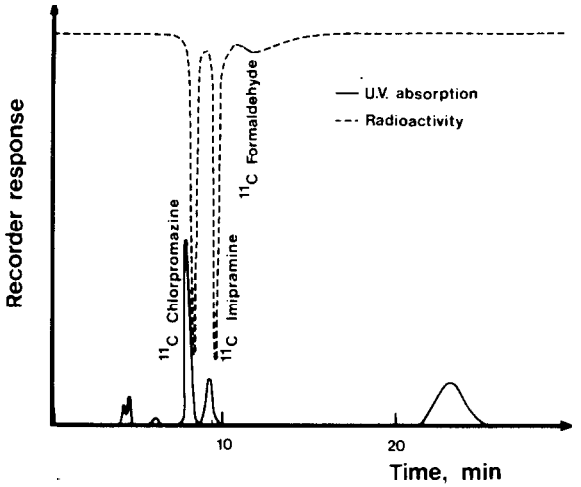


FIG. 4. Chromatograph of a synthetic mixture containing two radiopharmaceuticals: ^{11}C-chlorpromazine and ^{11}C-imipramine. Conditions described in the text.

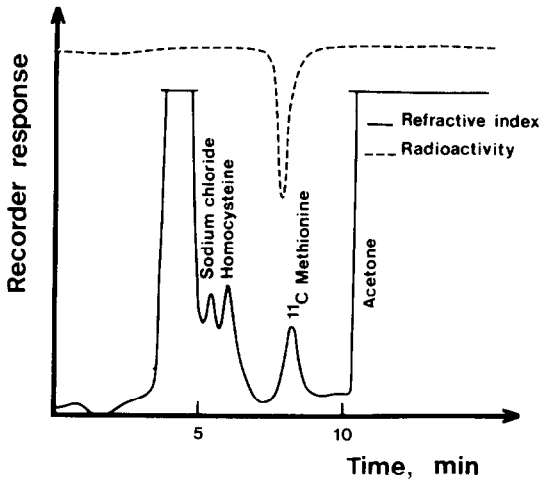


FIG. 5. Chromatograph of a synthetic mixture giving ^{11}C-methionine.

under vacuum in front of the ionisation chamber; the electrovalve is opened for filling and closed as soon as the required number of mCi has been transferred.

Results

The products obtained are chromatographically pure. Figure 3 shows a chromatogram obtained with a partial synthesis: a formaldehyde fraction which has failed to react, methanol left from incomplete catalysis and monodesmethyl derivative are well separated from the ^{11}C imipramine.

The RF values of the synthesized products check with those of standards. The same applies to the melting points and mass spectra when these examinations are performed.

All the samples tested (about 15 in all) have proved sterile and apyrogenic.

From a nitrogen target (pressure, 7 b, l = 40 cm) irradiated for 30 min with 20 MeV protons, intensity 25 μA, 70–120 mCi (average 95 mCi) of chlorpromazine, imipramine or nicotine are obtained 30–35 min after arrival of the radioactive carbon dioxide formed.

The chemical yield at the end of process (40–60%) is governed chiefly by the dehydrogenation reaction of methanol to formaldehyde.

The specific radioactivity at the time of use lies between 300 and 700 mCi/μmol (average 500 mCi/μmol), that of the formaldehyde obtained about 25 min earlier between 700 mCi/μmol and 1.6 Ci/μmol. The use of this automatic method improves by a factor 2 the specific activities obtained by the method described previously.[8]

Two kinds of molecule may also be prepared simultaneously by reaction of ^{11}C-formaldehyde on a mixture of two monodesmethyl derivatives in suitable proportions.

Figure 4 shows the chromatographic separation of ^{11}C-chlorpromazine and ^{11}C-imipramine (40 mCi each).

The quantities of ^{11}C-methionine obtained are of the same order or above (max 130 mCi), as are the specific activities (max 1.0 Ci/μmol).

The overall yield here is limited by that of the $I^{11}CH_3$ trapping in acetone.

The specific activity is better than for the other radiopharmaceuticals probably because, in this case, there is no isotopic dilution due to the decomposition of THF traces on the silver catalyst.[8]

Figure 5 shows the type of chromatogram obtained.

Conclusion

The method described allows about a hundred mCi of ^{11}C labelled chlorpromazine, imipramine, nicotine or methionine, chromatographically pure, sterile and apyrogenic, to be prepared in 30–35 min from 500 mCi to 1 Ci of $^{11}CO_2$ with no irradiation risk to personnel.

The reproducibility of the syntheses is good and the radiochemical purity and specific radioactivity of the product are known before the patient receives the injection.

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References

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