Synthesis and Radiopharmaceutical Preparation of (Ethylenediamine) (1-Carbon-11-Malonate) Platinum(II) for PET Studies

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Interest in the distribution, biotransformation, and mechanism of action of anticancer platinum complexes has led to the microscale, semi-automated and remote-controlled synthesis of (ethylenediamine) (1-[^{11}C]malonate) platinum(II) ([^{11}C]Ptenmal, EDMAL, JM40) from cyclotron-produced [^{11}C]cyanide. Carbon-11 cyanoacetate is produced by reacting [^{11}C]cyanide with bromoacetate. After hydrolysis, the resulting [^{11}C]malonic acid is purified and complexed to (diaquo) (ethylenediamine) platinum(II). Each step of the synthesis was optimized by studying the influence of different variables like reaction time and temperature, pH, necessary purification of intermediates, concentration and ratios of the reactants. Purification of the endproduct is achieved using preparative high performance liquid chromatography. The total incorporation of the [^{11}C]cyanide in the final product was 17–40%. After ~1 hr, ~40 mCi of [^{11}C]Ptenmal are produced in 10 ml sterile and isotonic dextrose solution ready for i.v. injection. The specific activity is ~ 200 mCi/μmol at EOB.

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Cisplatin (cis-diamine dichloro platinum(II), CDDP, JM 1) is one of the most active drugs against malignant tumors (*1–5*). The effectiveness, however, is limited by its toxicity causing nausea and vomiting, nephrotoxicity, neurotoxicity, and myelosuppression. In an attempt to develop new platinum analogs with less toxicity, many complexes have been synthesized. Some of the promising second-generation platinum drugs have a malonate ligand (*6–12*). The exact function and role of this chelating agent is still a matter of controversy and extensive investigation.

The incorporation of the positron-emitter carbon-11 (^{11}C) (20.4 min half-life) into radiopharmaceuticals is of great value for noninvasive metabolization and pharmacokinetic studies using positron emission tomography (PET) (*13–15*).

In view of these considerations, [^{11}C]Ptenmal is an interesting compound to elucidate the in vivo behavior of the dicarboxylate ligand. The aim of this work was therefore to develop a routine method for the radiopharmaceutical preparation of [^{11}C]Ptenmal by remote-controlled handling.

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MATERIALS AND METHODS

Carbon-11 Ptenmal Synthesis

Carbon-11 methane was produced by bombarding a gas target of nitrogen and hydrogen (95% : 5%) with 18 MeV protons. Possible traces of oxygen in the target gas were removed by passing the gas through an Alltech oxytrap filter. After batch production, the [^{11}C]methane was converted to [^{11}C]cyanide by catalytic reduction over platinum sponge heated to 900°C in the presence of ammonia, as previously described (*16*).

The produced [^{11}C]cyanide (1.5 Ci) was trapped in one ml water, which was then transferred to 2 ml of a concentrated sulfuric acid solution (vial A in Fig. 1). With a nitrogen flow, the H^{11}CN gas was filtered through glasswool (a) and trapped at 0°C in 500 μl of an aqueous sodium hydroxide solution (pH 10) containing 10 μmol sodium cyanide carrier (vial B). One hundred microliters of a solution containing 100 μmol of neutralized bromoacetic acid (adjusted to pH 8 with sodium hydroxide) were added and the reaction mixture heated at 95°C for 10 min. Subsequently, 300 μl of sodium hydroxide solution (3,000 μmol) were added and heating continued for 10 min. After cooling to 0°C, 500 μl of calcium chloride solution (3,000 μmol) were added and mixed. The slurry was

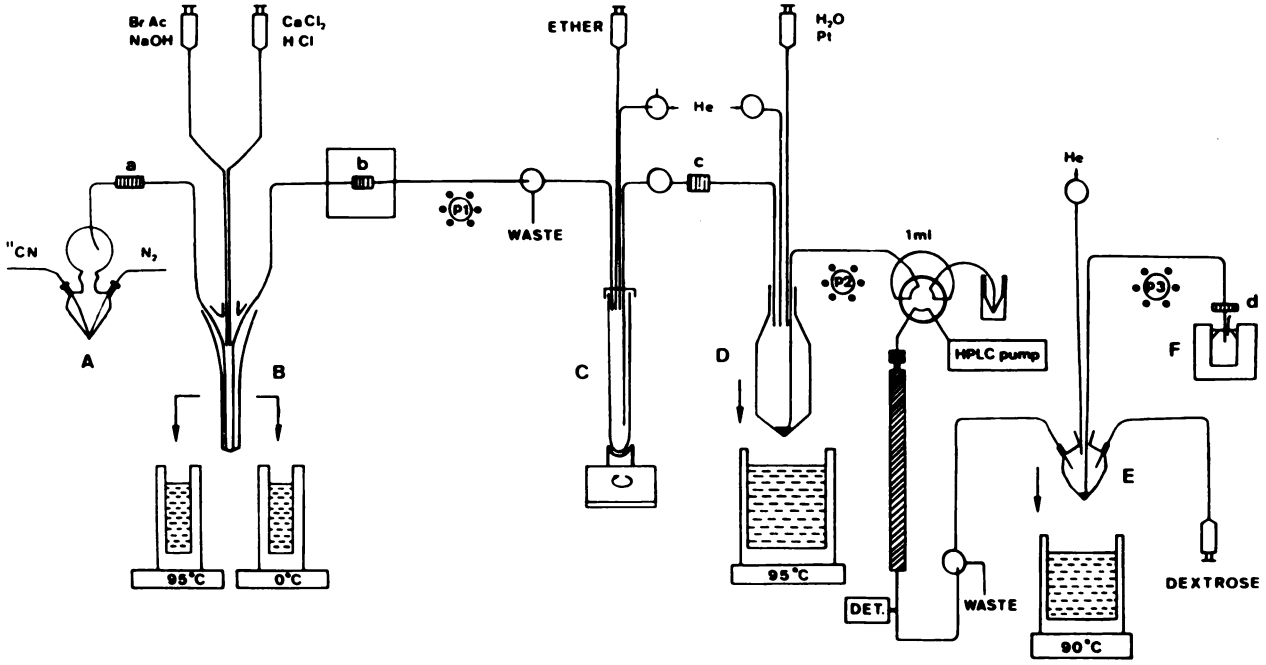

FIGURE 1
Production system in hot cell for [^{11}C]Ptenmal starting from [^{11}C]cyanide.

filtered (peristaltic pump 1, Fig. 1): the precipitate was retained on filter b while the filtrate was discarded to the waste. Five hundred microliters of hydrochloric acid solution (3,000 μmol) were added to vial B and sucked over filter b. When filter b was filled with the acid solution, the flow was stopped until the precipitate was completely dissolved with the aid of an ultrasonic bath. The resulting solution was then pumped over in vial C, 5 ml of ether were added and the mixture vortexed. The upper ether layer was pushed by a helium flow through a dehydrating filter c (containing anhydrous sodium sulphate) into evaporation vial D. This rapid extraction procedure was repeated five times. The ether fractions were evaporated to dryness at slightly elevated temperature by a helium flow. The residue was dissolved by adding 500 μl of water (pH 7.5). Subsequently, 500 μl of a freshly prepared solution of (diaquo) (ethylenediamine) platinum(II) (20 μmol Pt) was added and the mixture heated at 95°C for 3 min.

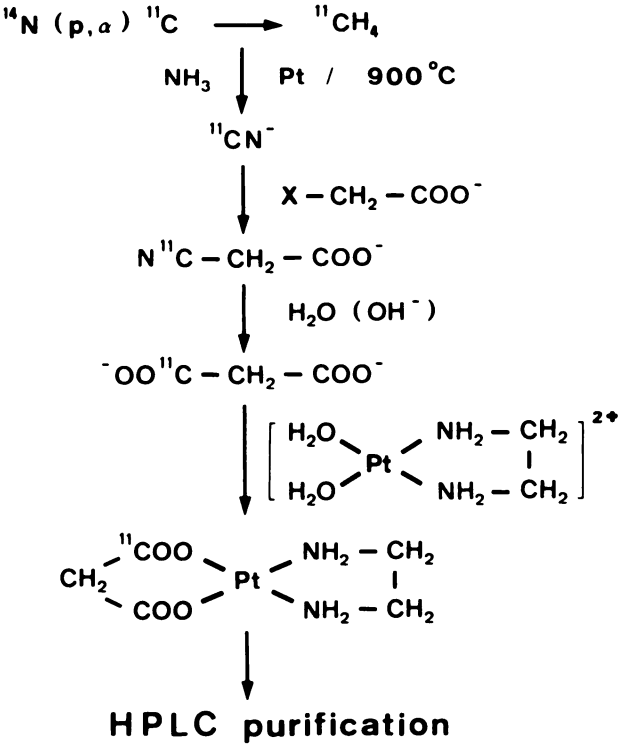

FIGURE 2
Reaction schema for the synthesis of [^{11}C]Ptenmal.

HPLC Purification

The pump (Waters M510) was equipped with stainless steel columns, connectors and frits. The detector system consisted of an uv detector (Pye Unicam LC3) set at 220 nm and a radioactivity counter (NaI(T1) detector, logarithmic scale). A Valco injector valve with a sample loop of 1 ml was used.

The preparative column (250 × 10 mm i.d.) was packed with RSil Silica (particle size 10 μm) and protected by a direct connect guard-column (Alltech). The mobile phase was ethanol/water (95:5 v/v) at room temperature. A flow rate of 5 ml/min was used.

The final solution in vial D was transferred in the sample loop with the aid of a peristaltic pump (p2) and injected onto the column. Detection involved both the absorbance at 220 nm and the on-line radioactivity determination. By switching a valve, the [^{11}C]Ptenmal was collected in vial E 10–12 min after the injection.

Radiopharmaceutical Preparation

Under a helium flow, the [^{11}C]Ptenmal eluate from the preparative HPLC column was evaporated at 90°C to a vol-

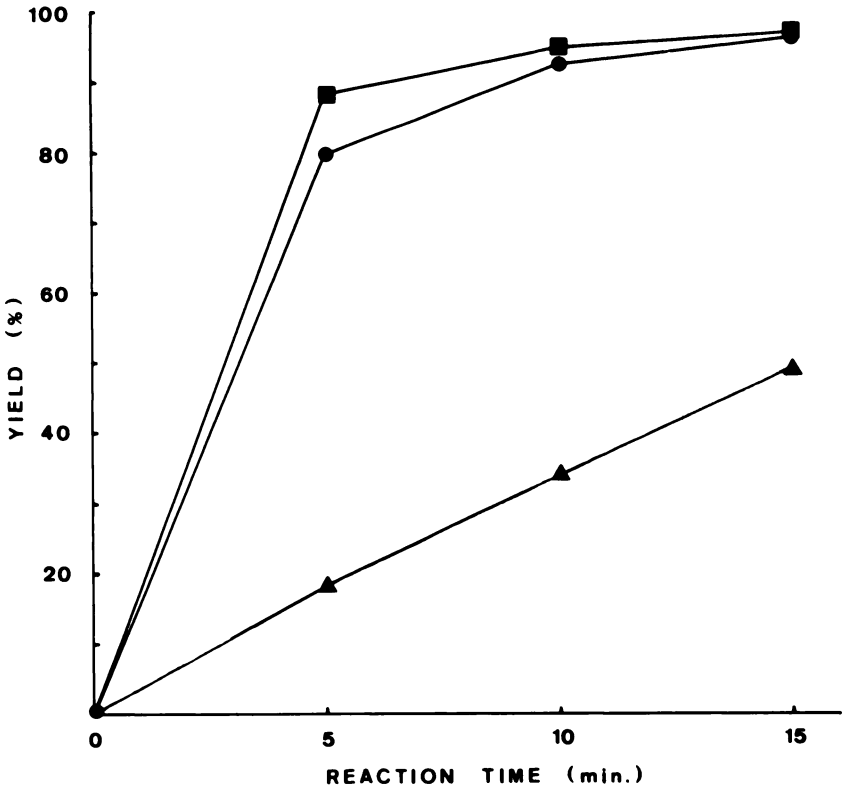

FIGURE 3
Conversion of sodium haloacetate to sodium cyanoacetate as a function of time. Neutralized haloacetic acid (100 μl, 100 μmol, pH8) was added to cyanide (500 μl, 10 μmol, pH 10). Reaction took place at 95°C. The leaving halogenide groups were chloride (▲), bromide (●) and iodide (■).

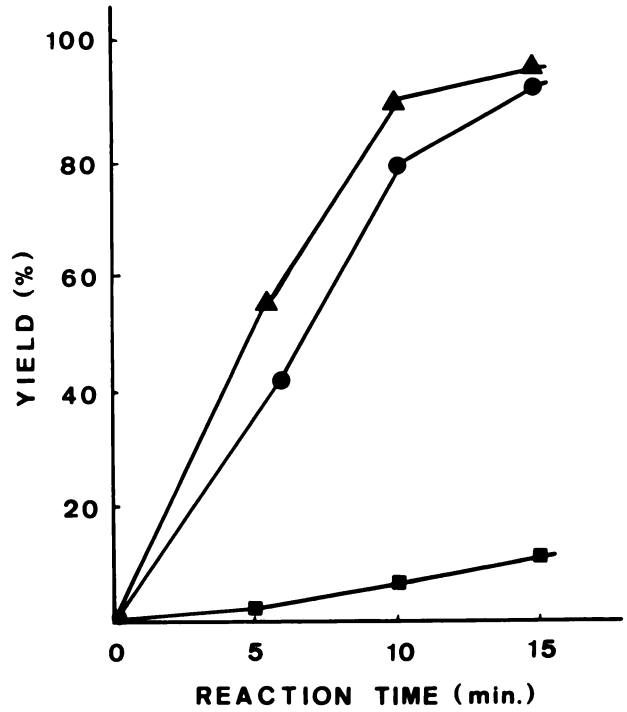

FIGURE 4
Effect of sodium hydroxide and reaction time on yield of sodium malonate at 95°C. The amount of sodium hydroxide is resp. 200 μmol (■), 1,000 μmol (●) and 3,000 μmol (▲).

ume of ~1 ml. Nine milliliters of a sterile, 5% m/v solution of dextrose were added and the mixture filtered (p3) under aseptic conditions through a bacterial filter (0.22 μm) (d) into a sterile injection vial (F).

Quality Control Procedures

Carbon-11 malonic acid was analyzed with ion-pair reversed-phase HPLC. The column used was a LiChrosorb RP C18 (5 μm particles, 150 × 4.6 mm i.d.) with an aqueous mobile phase containing 1 m*M* sodium phosphate and 0.5 m*M* tetrabutylammonium hydrogen sulphate, adjusted to pH 6.5. A sample loop of 20 μl and a flow rate of 2 ml/min. at 25°C were used.

Carbon-11 Ptenmal was chemically and radiochemically analyzed by high performance thin layer chromatography (HPTLC) (*17,18*) and by HPLC (*12,19*), using silica as stationary phase and acetonitrile/water as mobile phase. The influence of residual ethanol in the final solution was tested by hemolysis experiments, which were performed by adding 1.0 ml cosolvent (ethanol/aqueous dextrose solution) to 100 μl packed red blood cells (7.5×10^5 RBCs). Hemolysis time was 2 min. After centrifugation, the remaining RBCs were hemolyzed in 25.0 ml bidistilled water and the absorbance measured at 540 nm. The obtained values were transformed into percentages, which were regarded as a measure of intact RBCs. The final solution was controlled with the sterility and pyrogen tests of the European Pharmacopeia, which were done before the unit was applied for routine synthesis.

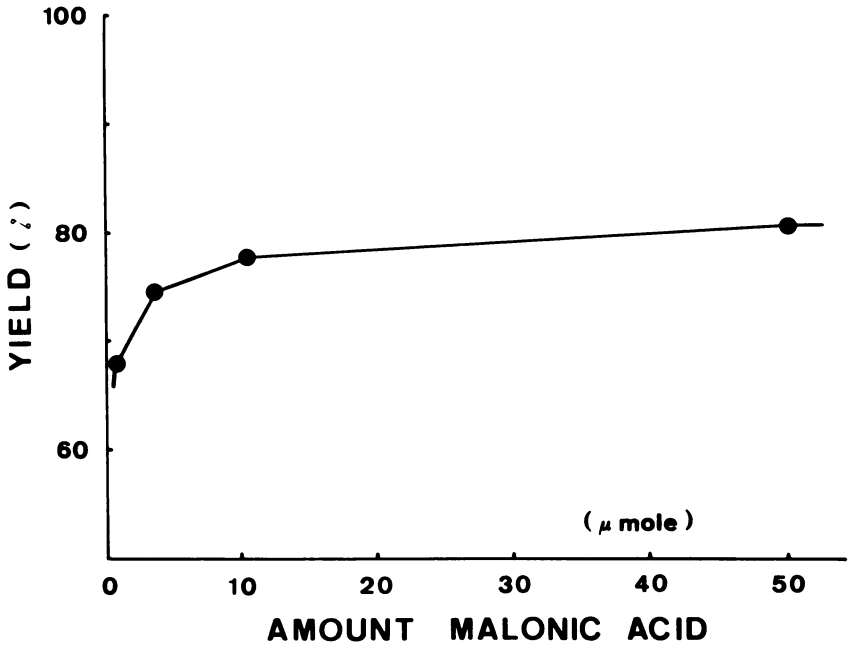

FIGURE 5
Recovery of malonate in the calcium precipitate as a function of the amount of sodium malonate present.

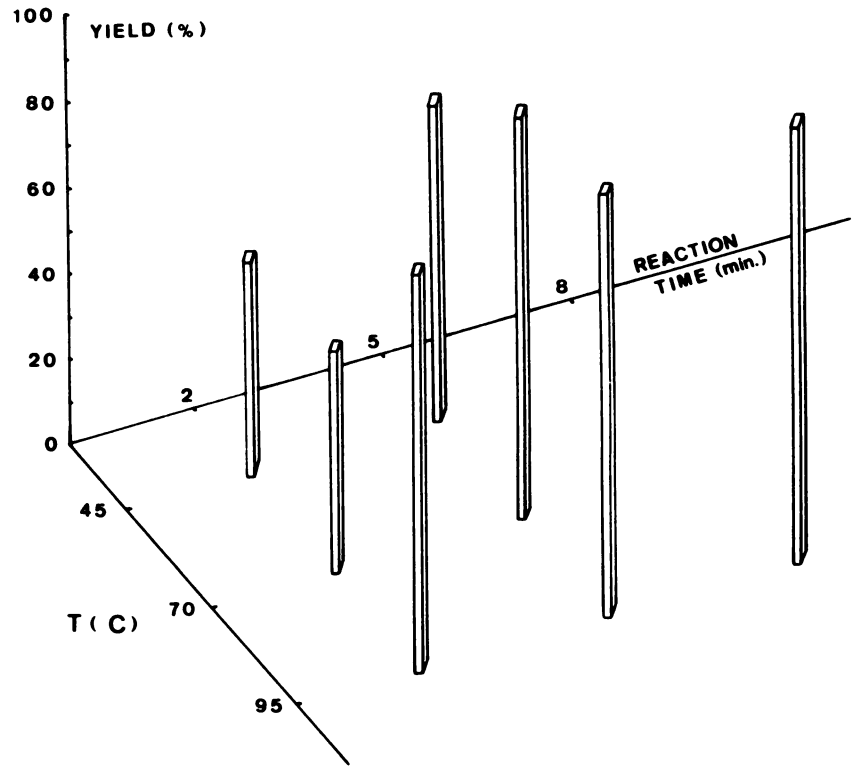

FIGURE 6
Effect of reaction time and temperature on Ptenmal yield. Five μmol malonic acid and 20 μmol (diaquo) (ethylenediamine) platinum(II) in a total volume of one ml were used.

RESULTS AND DISCUSSION

The remote-controlled setup used for the preparation of [^{11}C]Ptenmal in a hot cell is shown schematically in Figure 1. The corresponding reaction scheme is given in Figure 2.

Carbon-11 Cyanide Production

The yield and radiochemical purity of the [^{11}C]cyanide precursor depends on several factors (*16*). Some of these are difficult to control, e.g., the quality of the target material and the surface activity of the platinum catalyst. Two precautions were taken in order to elim-

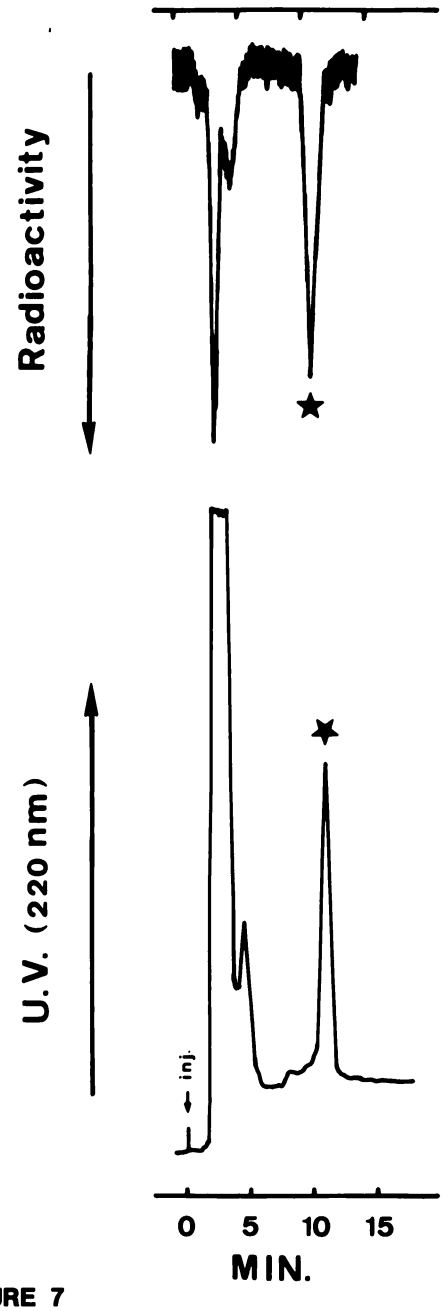

FIGURE 7
Preparative HPLC separation of a typical [^{11}C]Ptenmal reaction mixture. The [^{11}C]Ptenmal (*) elutes at ~11 min.

inate these difficulties and thus to enhance the yield and reproducibility of the synthesis. First, a rapid purification of the [^{11}C]cyanide precursor was achieved by the liberation of H^{11}CN in acidic medium. The glass-wool filter b (Fig. 1) between the condenser and the collection tube was necessary to break down the aerosol formed by the exothermic reaction of concentrated sulfuric acid and water. The radiochemical purity of [^{11}C]cyanide was investigated and proved to be pure by analytical HPLC (*20*). Second, 10 μmol carrier cyanide was added. In this way, not only an enhanced reproducibility of the synthesis was obtained, but also an increased recovery in the malonic acid purification step.

Carbon-11 Malonic Acid Synthesis

Carbon-11 malonic acid is prepared by the nitrile synthesis. Sodium cyanoacetate is obtained by treatment of sodium bromoacetate with sodium cyanide. Water is the solvent of choice because it dissolves both reactants and gives the highest yield. In ethanol the yield is only 25% of the yield in water. At a temperature of 95°C, the reaction is almost complete after 10 min. The bromo- and the iododerivatives are good candidates as starting reactant, as can be seen in Figure 3. The resulting nitrile is then hydrolyzed to malonic acid by the addition of alkali. The acid-catalyzed hydrolysis proceeded too slow to be useful. The rate of the base-catalyzed reaction is strongly dependent on the amount of sodium hydroxide added. As can be seen in Figure 4, as much as 3,000 μmol sodium hydroxide is needed for an almost complete conversion after 10 min reaction at 95°C. The synthesis optimization as well as the purification of the [^{11}C]malonic acid were controlled by HPLC as described in materials and methods.

Purification of Carbon-11 Malonic Acid

An extensive purification of malonic acid is required because aquated platinum species are highly reactive towards nucleophiles. Elimination of these competing reactive species is achieved by precipitation of malonic acid as calcium malonate, acidification, and extraction with diethyl ether. Other preparative purification methods like HPLC, ion-exchange and liquid-solid extraction were tried but found to be less satisfactory. The yield of calcium [^{11}C]malonate in the precipitate is ~ 80%. However, this value is dependent on the amount of malonic acid present: the yield rapidly decreases when <10 μmol are present (Fig. 5). By this precipitation step, the excess of bromoacetic acid and its hydrolyzed product, e.g., hydroxyacetic acid, is eliminated.

The precipitate, which consists of calcium hydroxide and calcium malonate, is dissolved in hydrochloric acid. The resulting solution has a very high salt concentration which facilitates the extraction of malonic acid. Several extraction solvents have been tried. The halogenated hydrocarbons give significantly lower yields, while ethylacetate was difficult to evaporate quickly. So, ether was the best compromise between extraction yield and evaporation time. The isolated ether fraction however contains some dissolved water and chloride ions. If the ether is not dried, a yellow precipitate—probably $PtenCl_2$—is formed when the residue reacts with the aquo platinum complexes. Anhydrous sodium sulfate was used for drying the ether, which can then be easily evaporated. The resulting residue was checked by HPLC and found to be radiochemically pure. Traces of nonradioactive hydroxyacetic acid were also present.

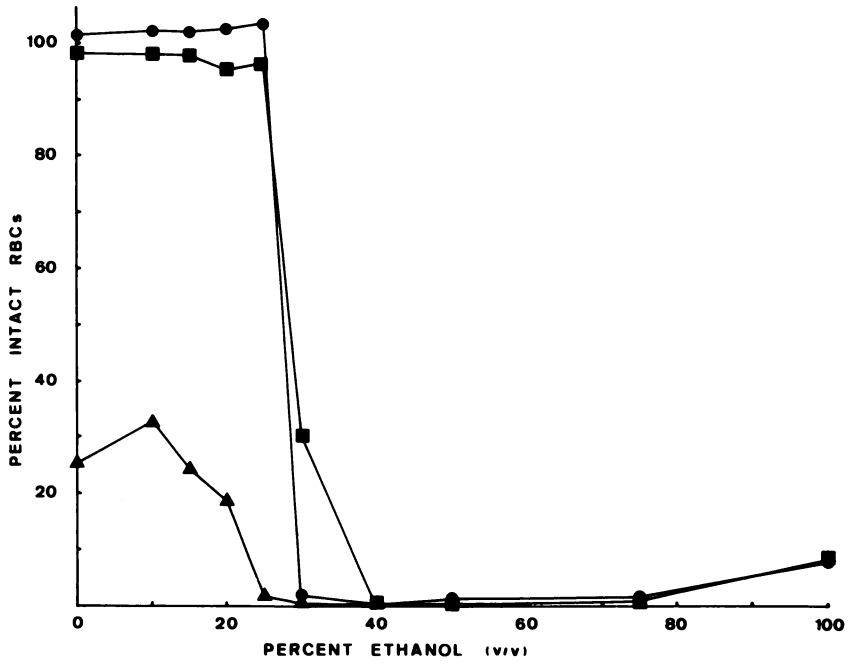

FIGURE 8
Effect of ethanol concentration on the hemolysis of red blood cells. The aqueous solution contains resp. 2.5% (▲); 5.0% (■); and 10.0% (●) dextrose.

Synthesis of Carbon-11 Ptenmal

The [^{11}C]malonic acid residue, obtained by evaporation of the etheric phase, is dissolved in water of pH 7.5. Alkaline or strong acidic reaction conditions significantly decrease the reaction yield. An excess of (diaquo)(ethylenediamine)platinum(II) is added, because of the presence of traces of competing nucleophiles, like hydroxyacetate. Reaction time and temperature were simultaneously optimized. As can be seen in Figure 6, reaction at 95°C was almost quantitative after only 3 min. These experiments were controlled by HPLC and HPTLC (*12,17–19*). Comparable results were obtained.

Purification and Radiopharmaceutical Preparation of [^{11}C]Ptenmal

Because of the high toxicity of possible side products and intermediates of the synthesis, e.g., aquated platinum compounds, HPLC was used to purify the final reaction mixture. Preparative chromatography using RSil Silica gave good separation with acetonitrile/water as mobile phase. However, the presence of acetonitrile in the mobile phase complicates the final purification, since this highly toxic solvent must be carefully and completely removed. As a consequence, ethanol was used as the organic component of the mobile phase. With a mixture of 95% ethanol and 5% bidistilled water, the radioactive ptenmal was collected between 10 and 12 min after injection, which is acceptable for ^{11}C-labeled compounds. An example of a typical chromatogram is shown in Figure 7. The purification was controlled by double detection: a NaI(Tl) detector for measuring the annihilation radiation and a uv detector for carrier detection. Since the [^{11}C]Ptenmal is to be used for animal and human studies, a solution suitable for i.v. injection must be prepared. A neutral compound, dextrose, was chosen to adjust the isotonicity because of the possible reactions of platinum complexes with nucleophiles like physiological saline. The volume of the eluent fraction of the HPLC containing [^{11}C] ptenmal was reduced under a stream of helium at 90°C. The influence of residual ethanol and dextrose aqueous concentration on hemolysis was tested utilizing a cosolvent to blood ratio of 10:1 (*21*). As can be seen in Figure 8, no hemolysis is observed with <30% ethanol and with isotonic (5% m/v) or higher concentrations of dextrose in the aqueous solution. Therefore, the addition of 9 ml of a 5% m/v dextrose solution to ∽ 1 ml ethanolic residue resulted in a nontoxic, nonhemolytic solution. The fact that no dried residue of [^{11}C]Ptenmal is needed enhances the rapidity of the procedure because of less evaporation time and easier dissolution of the labeled compound in the dextrose solution.

The final solution was analyzed by analytical HPLC (*12*). This gave only one peak in the chromatogram corresponding to Ptenmal. We therefore conclude that the [^{11}C]Ptenmal obtained is radiochemically pure.

Yield and Specific Activity

Table 1 shows the distribution of ^{11}C activity. In a typical experiment, 1.5 Ci [^{11}C]cyanide was introduced into the synthesis, yielding about 40 mCi [^{11}C]ptenmal with a relative standard deviation of 12% (n = 3). The total synthesis time, including the purification and preparation of an injectable solution, takes about three to four half-lifes. A carrier amount of ∽ 3 μmol was obtained, which is below the dose given in chemotherapy, giving a specific activity of 200 mCi/μmol at EOB.

TABLE 1
Distribution of ^{11}C Activity*

Sulfuric acid solution (vial A)	15%
Waste fraction (after p1)	20%
Aqueous layer (C)	10%
Filter c	8%
Not retained HPLC waste fraction	16%
Ptenmal fraction (F)	31%

*n = 3.

The radioactive Ptenmal isotonic dextrose solution was found to be sterile and free from pyrogenic material, and could therefore be applied for i.v. injections in humans to perform positron emission tomography studies.

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