Journal of Labelled Compounds — April-June — Vol. III, No. 2

Preparation of 3-5 Dipropionilamino 2, 4, 6, Triiodo Benzoic Acid I-131

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Received on 7th November 1966

SUMMARY

A method is discussed for the labelling of Diprocon by interchange between ^{131}I and inactive Diprocon, obtaining a radiochemical yield over 95% in only five minutes.

The effect of pH, temperature and molar relation between the reactants on the radiochemical yield is determined as well as the maximum sterilization time and the optimal storage conditions for the labelled molecule.

INTRODUCTION.

There is abundant literature about the use of several iodine and N-acyl substituted benzoic acid derivatives as contrast mediums for ascendent urography, brain angiography, excretory pielography, etc. [1, 2, 3, 4, 5, 6].

Through the revision of the literature, we found some information differing in the relative behaviour of each of these compounds, regarding their properties and their toxicity [4, 5, 6, 7, 8, 9, 10, 11, 12, 13].

Nevertheless, it is generally accepted that these compounds are toxic only above certain levels. As already known, the use of these radiographic contrast mediums is based on their achieving a certain concentration in the vase under study and in the case of kidney, it is based on the high extraction level this organ shows for the above mentioned substance.

To obtain nitid radiographic films it is necessary to give the patient a quantity of drug calculated considering both the nitidity of radiographic film desired and the substance toxicity.

There is experience in the use of some of these radioactive products giving satisfactory results [14, 15].

In 1959, Liebster *et al* [16] prepared 3-5 diacetyl amino, 2-4-6 triiodo benzoic acid ^{131}I methyl glucamate (Urografin) by interchange with ^{131}I iodide and obtained a radiochemical yield of 66% in 12 hrs. 100° C and Taplin [14], in the same year, used the 2,5 dipropionilamino 2,4,6 triiodo benzoic acid (Miokon, diprotrizoic acid) labelled with ^{131}I.

The beginning of the production of 2-5 dipropionil amino 2,4,6 triiodo benzoic acid (Diprocon) in our country, impelled us to study the optimal conditions for its labelling with ^{131}I and we obtained a radiochemical yield of above 98%.

From the practical point of view the use of this radioactive product would be limited to the study of kidney function and the target of the present work is to describe the preparation technique by interchange, the influence of different variables on the radiochemical yield and the stability of the radioactive molecule to the action of light and temperature.

EXPERIMENTAL PART.

Labelling technique.

2 ml of 3-5 dipropionilamino 2-4-6 triiodo sodium benzoate (0.05 mM/ml) in acetic acid-sodium acetate buffer pH 4.6 plus 1-10 mCi ^{131}I sodium iodide, carrier and reductor free (CEN Saclay, France) and 1 drop H_2O_2 20 vol. are placed in a glass stoppered centrifuge tube and put in a water bath for 5 minutes.

The tube is removed from the bath, cooled in an ice bath, centrifuged and reduced with a small excess 1 M sodium tiosulphate — Radiochemical yield : 98%.

If necessary, it can be reprecipitated with 1 N ClH, cooled and centrifuged (discarding mother liquor). Then, it is dissolved in 0.1 N sodium hydroxide and adjusted to pH 7.2. It is sterilized with water vapor during 30 minutes.

Control.

a) Radioactivity was measured in an ionization chamber (Techniatomic Model TCS 100, solutions calibrator).

b) Sterility and absence of piretycs are determined by the usual methods.

c) Inorganic radioiodine is detected by paper electrophoresis in Whatman 3MM paper (10 V/cm, 2-4 mA) in acetic acid-sodium acetate buffer (0.08 M; pH 5.5) during 90 minutes.

Diprocon migrates between 3-5 cm and iodide 10-12 cm. The dried papers are evaluated radiometrically in a Packard radioscanner, model 7200 and the resulting areas, determined by weight.

It can be also determined by thin layer chromatography and subsequent radiometric evaluation with a Radioscanner [17].

Calculations.

The percentage of inorganic radioiodide is obtained from the following equation :

$$\%\,I = \frac{\text{Weight of the iodide area } (a_2) \times \text{range } (r_2)}{a_1 r_1 - a_2 r_2} \times 100$$

The time constant (RC), colimation (e) and velocity (v) of the paper and register chart (as they move synchronously) are chosen. The residence time (T) under the detection unit has been calculated in each case, the minimum equal to 1.5 times the equilibrium time (t_0), considering [18].

$$to = RC(0.394 - 1.15 \log 2.A.RC)$$

where A represents the maximum activity to be measured, determined by previous attempts.

Example.

0.1 mM Diprocon-pH 4,5,6 min-100° C-50 0.001 N KI (Exp. No. 79).

Time constant (RC)	1 sec
Collimator (e)	2.5 mm
Diprocon range (r_1)	3×10^5 c/min
Iodide range (r_2)	3×10^4 c/min
Chart width (h)	150 mm
Register chart area per unit weight (p)	22.7 mm²/mg
Max. activity to be measured (A)	1.2×10^5 c/min

$$t_0 = 1 \text{ sec}(0.394.1.15.\ \log 2 \times 1.2.10^5 \text{ c/min.min/60}) = 4.535 \text{ sec}$$

$$\frac{e}{V} = 1.5\ t_0$$

$$V = \frac{e}{1.5\ t_0} = \frac{2.5 \text{ mm} \times 60 \text{ sec/min}}{1.5 \times 4.53 \text{ sec}} = 22 \text{ mm/min}$$

Velocity used : 20 mm/min
Diprocon area weight (a_1) = 64 mg
Iodine area weight (a_2) = 115 mg

$$\%\,I = \frac{115 \text{ mg}.3 \times 10 \text{ c/min}}{(64 \text{ mg}.3 \times 10^5 \text{ c/min}) - (115 \text{ mg} \times 3 \times 10^4 \text{ c/min}} \times 100 = 15.3$$

The detection units are two windowless, 4π geometry Geiger tubes with gas flow, which allow measurement of sources up to 4×10^5 c/min

without error by coincidence. This was previously determined following the ^{128}I decay with the same counting geometry.

Influence of different variables.

The influence on the radiochemical yield following the change of several variables was studied (Tables I, II, III, IV).

The general procedure exposed to the labelling technique was applied, with the following precautions :

a) When the test tube was taken off the water bath it was placed in an acetone-dry ice mixture to stop the reaction.

b) After reduction with excess 1 M sodium tiosulphate, the state of the reaction was determined by paper electrophoresis, according to the technique and calculations discussed. The results obtained are stated below. In the graphics, the deviation resulting of averaging the different experiences has been fixed.

Stability.

Periodic determinations on two series of patterns originally free of radioactive iodide were carried out at different pH values and sterilization times in order to determine the stability of the labelled molecule.

One of the series was exposed to sunlight at room temperature and the other was kept at darkness at 4° C.

We used Diprocon ^{131}I solutions of 10 mCi/mM and 0.01 mM/ml at pH 6, 7.2 and 8.2 and sterilization times of 30 and 60 minutes.

All the patterns became slightly coloured after some time. Nevertheless, 15 days later only those solutions at pH 8.2 liberated radioiodine in considerable quantities e.g. 8% those exposed to sunlight and 3% those kept at darkness at 4° C.

Finally an increase of inorganic iodine in the solutions sterilized for a longer time was observed.

CONCLUSIONS.

Experiments carried on indicate that the working pH is only limited by the solubility of the drug and the acidity necessary to achieve the oxidation reaction of I by hydrogen peroxide.

A strong mass action is necessary to obtain a yield over 95% (2×10^4) moles of Diprocon per iodine atom gram.

Solution sterilized up to 1 hour and kept at pH 6-7.2 stays practically unaltered for at least 15 days.

The labelled product is useful for studies in man and it has been used for that purpose at the Nuclear Medicine Center (Argentine).

TABLE I. pH influence on the radiochemical yield (^{131}I Na carrier and reducing agent free — 0.1 mM Diprocon — 5 min — 100° C).

pH 3.7	99.3% — 0.1
pH 4.6	98.3% — 0.3
pH 5.5	98.3% — 0.2
pH 6.0	97.9% — 0.4
pH 6.8	77.0% — 1.6
pH 7.2	27.0% — 0.1

TABLE II. Reaction time influence on the radiochemical yield (^{131}I Na carrier and reducing agent free — 0.1 mM Diprocon — pH 4.6 — 100° C).

10 minutes	98.5% — 0.2
5 minutes	98.3% — 0.3
4 minutes	97.9% — 0.2
3 minutes	96.7% — 0.9
2 minutes	92.7% — 1.8
1 minute	76.9% — 0.5

TABLE III. Reactants concentration influence on the radiochemical yield (^{131}I Na carrier and reducing agent free — pH 4.6 — 5 min —100° C).

0.001 mM	63.2% — 1.9
0.01 mM	94.3% — 0.8
0.05 mM	97.4% — 0.2
0.1 mM	98.3% — 0.3
1.0 mM	99.9% — 0.1

TABLE IV. Reactants concentration influence on the radiochemical yield (0.1 mM Diprocon — pH 4.6 — 5 min)

carrier free	98.3% — 0.3
0.0001 N	98.0% — 0.5
0.001 N	85.2% — 0.7
0.01 N	30.0% — 1.9
0.05 N	2.0% — 0.3
0.1 N	0.0%

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