KINETICS AND MECHANISM OF THE NUCLEOPHILIC EXCHANGE REACTION OF IODIDE-131 WITH 15-(PARA-IODO- PHENYL) PENTADECANOIC ACID

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SUMMARY

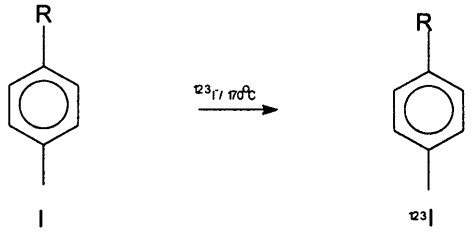
A procedure for labelling p-IPPA with ^{131}I by iodine isotope exchange in ethanol using benzoic acid was described. The method results in an overall radiochemical yield of more than 80 % within 50 min. at 170°C. The specific activity of the final product was 10 mCi/mg .The reaction conditions were investigated using Cu(I) in acetic acid, pivalic acid, ammonium acetate and ammonium sulfate. The activation energy of the reaction was calculated to be E= 33.54 Kcal/ mol. Quality control of the product was performed by means of high pressure liquid chromatography HPLC.

Key words : 15- (para-[^{131}I]- iodophenyl)-pentadecanoic acid, No- carrier- added [^{131}I] Activation energy, High performance liquid chromatography

Introduction

Since free fatty acids are the principal energy source for the normally oxygenated myocardium, the use of iodine labelled fatty acid analogues is an attractive approach for myocardial imaging, and suitable as radiopharmaceuticals(1-4)

In order to simplify the preparation of the promising radiopharmaceutical p-^{123}IPPA for myocardial scintigraphy, an iodine- isotope exchange method for "cold" p-IPPA with radioiodide was suggested as characterized by the following reaction (5), equation 1 .



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"cold"- p-IPPA 95%

The preparation of radioiodinated p-IPPA by iodine-isotope exchange

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This method enabled a 95 % radiochemical yield of ^{123}IPPA within 1h which could be used without the application of any complicated separation techniques.

Copper(I) salts have proven useful for the catalysis of isotopic exchange of radiobromide and radioiodide onto aromatic rings (6,7), and for the isotopic exchange labelling of radioiodinated steroides (8). *In situ*-generated copper(I) has been used for the low-specific activity synthesis of N-isopropyl-p-[^{123}I]iodoamphetamine (9), 15-(p-[^{123}I]iodophenyl)-9-methyl pentadecanoic acid (10), as well as [^{123}I]iodohippuric acid and other radioiodinated tracers (11). Many examples have appeared in the literature showing that various copper salts have facilitated exchange aryl iodides (12).

15-(4-iodophenyl)-pentadecanoic acid has been radioiodinated with ^{123}I in a melt of benzoic acid at 170°C in 95% (13). A simple method for preparing [^{123}I]mIBG in high yield at high radiochemical purity in presence of ammonium sulfate has been reported (14)

In this work we have investigated the labelling conditions suitable for the rapid isotopic exchange of ^{131}I- for- I in p-IPPA; variables included temperature, the effect of pivalic acid, ammonium acetate and ammonium sulfate content in the dry state, also the effect of solvents, Cu (I) in acetic acid on the radiochemical yield of p-^{131}IPPA have been studied. In order to prepare p-^{131}IPPA more effectively, it is necessary to carry out a kinetic study to obtain more reliable reaction conditions. Quality control of the product was performed by HPLC.

Experimental

Materials

Pure 15-(p-iodophenyl)-pentadecanoic acid was purchased from Cambrian Chemicals Co. Na^{131}I (nca) was locally produced in our laboratory. All other reagents used throughout this work were of analytical grade and used as received without any further purification.

Labelling Procedure

Labelling of 15-(p-iodophenyl)-pentadecanoic acid (p-IPPA) with radioiodide by isotopic exchange was carried out (13), in a V shaped reacti-vial which could be tightly closed by a screw cap. In this vial 1 mg of p-IPPA and 3 mg of benzoic acid dissolved in 0.1 ml ethanol were added. Thereafter 10µl of 0.1 N NaOH solution with about 3.7 MBq $^{131}I^-$ iodide was added. The solvent was evaporated on a vacuum line. The reacti-vial was closed and heated at 170°C in an oil bath. The experiment was repeated several times to study the effect of heating time (2- 60 min.). The reaction was stopped by cooling the vial in an ice bath.

Chromatography

HPLC analysis

The radiochemical yield of the p-^{131}IPPA was determined by direct injection of 5-10 µl of the reaction vessel contents into HPLC Shimatzu Model, LC- 9A pump, with a Rheodyne injector (Syringe Loading Sample Injector-7125) and u.v. spectrophotometric detector Shimatzu SPD-6A. The radioiodinated p-^{131}IPPA was separated with stationary phase comprised of analytical column RP-18 (250 x 4 mm , 5 µm) Lichrosorb, ODS from Merck and a mobile phase consisting of methanol : water : acetic acid (95 : 3 : 2) at a flow rate 1ml / min. (12). The separated p-^{131}IPPA were collected in vials and the radioactivity was determined in a well type NaI (Tl) crystal. As shown in Fig. 1 the final reaction mixture contains two products : major product p-^{131}IPPA at retention time t_r (t_r = 18.9 min.), free $^{131}I^-$ (t_r = 1.8 min.).

Thin Layer Chromatography

Radiochemical yields of the exchange reacations were estimated by thin layer chromatography (TLC) on aluminum sheets silica gel 60 using diethyl ether as developing solvent (13). A volume of 1-2 µl of the final solution was put on the chromatographic plate which was previously impregnated with $Na_2S_2O_3$ (20 m/ml) to inhibit the oxidation of radioiodide to a volatile form, then chromatographed for about 60 min. (corresponding to 10 cm migration of the solvent). The strips were removed , dried and cut into 0.5 cm and assayed for radioactivity using SR-7 gamma counter. The radioiodide and the radioiodinated p-^{131}IPPA had R_f values of 0.04 and 0.91 respectively.

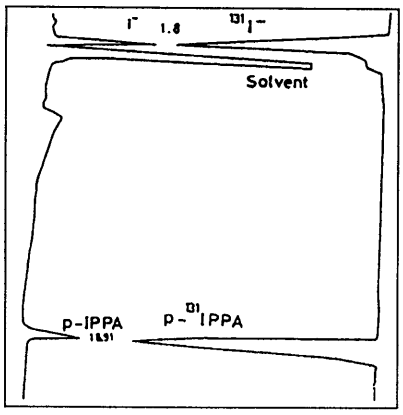


Fig.1 : separation of the final product p-^{131}IPPA by high performance liquid chromatography (HPLC). Lift trace : UV signal . Right trace : Radioactive signal

Results

Effect of temperature

To study the influence of temperature on the radiochemical yield of p-^{131}IPPA, the exchange reactions in the dry state (after evaporation of ethanol) were performed with 1 mg p-IPPA and 3 mg of benzoic acid in ethanol and 10 µl Na^{131}I. As shown in Fig. 2, a labelling. yield of ≈80% was obtained by heating the reaction mixture for 50 min. at 170°C. At lower temperatures the exchange rate was rather low in comparison with that at 170°C. The radiochemical yield was very poor at 100°C

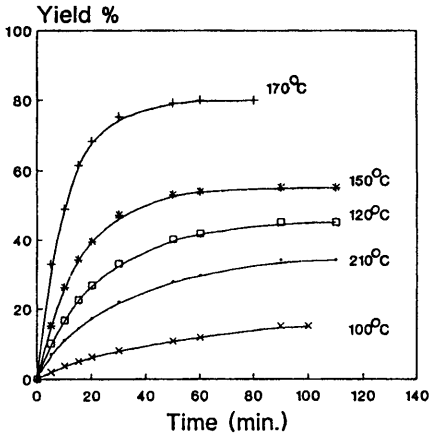


Fig. 2 : Variation of the radiochemical yield of p-^{131}IPPA as a function of reaction time at different temperatures in dry state [3.7 MBq Na ^{131}I + 1 mg p-IPPA + 3 mg benzoic acid]

Effect of pivalic acid content

In order to investigate the catalytic effect of pivalic acid on the exchange reaction in the dry state, p-IPPA has been radioiodinated with ^{131}I⁻ in the presence of the acid at 170°C for different time periods (0-120 min). As could be seen from Fig.3, the incorporation of radioiodide increases with the amounts of pivalic acid.

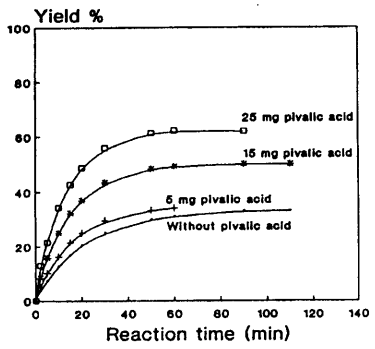


Fig. 3 : Variation of the radiochemical yield of p-^{131}IPPA as a function of reaction time at different quantities of pivalic acid [3.7 MBq $Na^{131}I$ + 1mg pIPPA + Xmg pivalic acid] at 170°C.

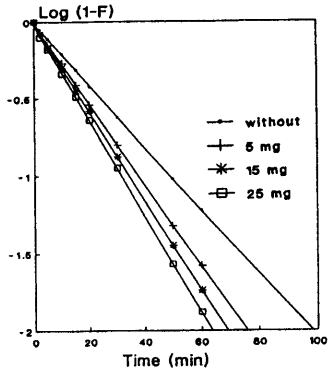


Fig .4: Variation of log (1-F) as function of reaction time at different quantities of pivalic acid [3.7 MBq $Na^{131}I$ + 1mg p-IPPA + Xmg pivalic acid] Temp. = 170°C.

The reaction was complete in 1 h with 63 % radiochemical yield. These results imply that in addition to the solvent role of pivalic acid, it has also a catalytic influence on the exchange reaction. Moreover, the steric hinderance associated with pivalic acid also decreased the likelihood that it becomes a reactant or promoter of side reactions under the conditions of the exchange reaction (15). The plots of Log (1-F) vs time, when using different amounts of pivalic acid, at a constant concentration of p-IPPA (0.011M) at 170°C are given in Fig.4. These plots are linear for all studied concentrations.

Effect of ammonium acetate

In order to reduce the reaction temperature from 170 to 120°C, the isotopic exchange reaction between p-IPPA and $Na^{131}I$ in the dry state was performed in presence of various amounts of ammonium acetate and a fixed amount of benzoic acid (3mg) at 120°C within 60 minutes. It could be observed from table 1 that a quantity of 10 mg of ammonium acetate is sufficient to obtain 60 % radiochemical yield of p-^{131}IPPA, compared with a yield of only ≈ 41.74% in absence of ammonium acetate.

Table 1 : Varation of radiochemical yield of 15-^{131}IPPA as a function of various amounts of ammonium acetate witin 60 min. at 120°C

Quantity of ammonium acetate (mg)	Radiochemical yield %
0	41.74
3	50.34
5	55.00
10	60.30
15	44.30
25	33.90

Influence of ammonium sulfate

The influence of ammonium sulfate content on the radiochemical yield of p-^{131}IPPA in the dry state is shown in table 2 from which it could be observed that the radiochemical yield of p-^{131}IPPA increases from ≈ 30 to 50 %, with the amount of $(NH_4)_2SO_4$ being increased from 0 to 10 mg within 60 min at 170°C.

Table 2: Varation of radiochemical yield of 15-^{131}IPPA as a function of various amounts of ammonium sulfate witin 60 min. at 170°C.

Quantity of ammonium sulphate (mg)	Radiochemical yield (%)
0	31.00
5	38.50
10	50.00
15	18.70
25	14.60

Influence of solvent :

Radioiodination of p-IPPA with Na^{131}I in some organic solvents such as ethanol , dioxane , acetic acid and dimethyl sulphoxide (without using benzoic acid) was examined as shown in Fig. 5. From this comparative study, it was deduced that ethanol is the solvent of choice for the labelling of p-IPPA with $^{131}I^-$, being as well superior to all other solvents used. A maximum radiochemical yield of ≈ 60 % is achieved within 100 min for p-^{131}IPPA at 170°C .

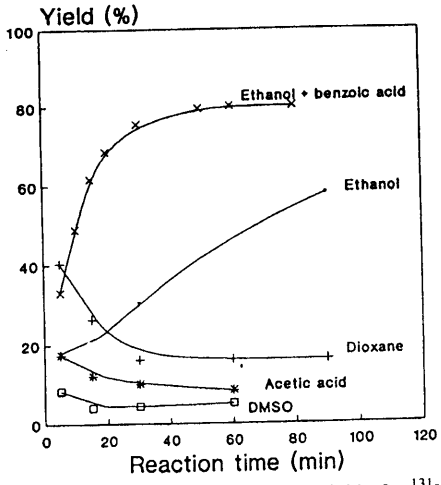


Fig. 5 : Variation of the radiochemical yield of p-^{131}IPPA as a function of reaction time using different organic solvents [3.7 MBq Na^{131}I + 1mg p-IPPA in 200 µl] at 170°C.

Influence of Copper (I) chloride concentration

Fig.6 illustrates the relationship between the reaction time and the radiochemical yield of p-^{131}IPPA using different amounts of CuCl in acetic acid at 170°C. NCA incorporation of radioiodide occured rapidly using 200 µg (10 m mol) with a radiochemical yield of 70 % within 30 min. Higher radiochemical (77 %) yields were achieved when the reaction time was extended from 60 to 120 minutes.

It is clear that the presence of CuCl is essential for efficient nca radioiodination to take place. The corresponding production of p-^{131}IPPA under these conditions in the absence of CuCl was negligible (<10 %). Lower concentrations of cuprous chloride led to a decrease in the labelling yields ≈ 30 % using 50 µg (2.5 m mol) wthin 60 min. This decrease is probably due to insufficient interaction between copper species and aromatic substrate (16). The relation of Log (1-F) against time was plotted at different CuCl concentrations as shown in Fig.7, which indicates that the rate of isotopic exchange was enhanced by increasing the amount of CuCl.

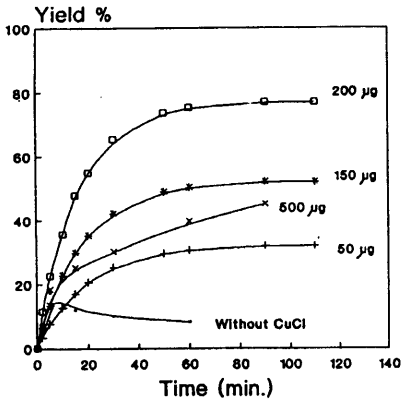


Fig. 6: Variation of the radiochemical yield of p-^{131}IPPA as a function of reaction time using different quantities of cuprous chloride (CuCl) [3.7 MBq $Na^{131}I$ + 1mg p-IPPA in acetic acid (200 µl)+ Xµg CuCl] Temp. = 170°C.

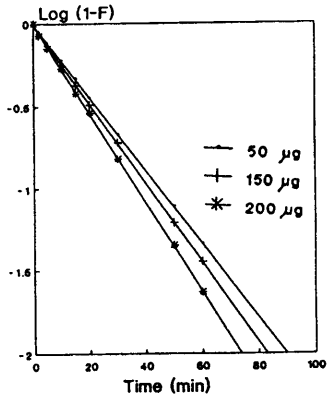


Fig .7: Variation of log (1-F) as function of reaction time and different quantities of cuprous chloride (CuCl) [3.7 MBq $Na^{131}I$ + 1mg p-IPPA in acetic acid (200 µl) + Xµg CuCl] Temp. = 170°C.

CuCl/ p-IPPA molar ratio.

The influence of copper (I) chloride concentration on the radiochemical yield of p-^{131}IPPA was studied, not only to maximize radiolabelling yields, but also to minmize damage to the substrate molecule by copper ctalyzed side -reactions (17,18). The relationship between the relative CuCl/ p-IPPA molarities and the radiochemical yield of p-^{131}IPPA is showen in table 3

Table 3 : The relationship between the relative CuCl/ p-IPPA molarities and radiochemical yield, where concentration of p-IPPA is constant, 0.011M

Quantity of CuCl (µg)	Concen. of CuCl (mM)	CuCl/p-IPPA molar ratio	percent yield (%)
50	2.4	0.22	30.54
100	4.9	0.45	40.00
150	7.4	0.67	50.15
200	9.8	0.89	75.19
220	11	1.00	73.00
300	14.7	1.34	60.00
500	24.8	2.25	39.60

Discussion

The preparation of p-^{131}IPPA via an iodine isotope exchange method for cold p-IPPA with radioiodide is suggested as characterized by equation 1. The most influent single factor affecting the labelling yield was temperature. According to Fig 2 the reaction was fast at 170°C. The temperature decrease from 170 to 100°C within a 30min reaction time lowered the yield of p^{131}IPPA from ~80 to <10% . This may be mainly attributed to the insolubility of the fatty acid at this temperature. At higher temperatures (210°C), the yield still diminished. This behaviour

can perhaps be explained by a thermal decomposition of the product competing with its formation (19). The optimum temperature was therefore considered to be 170°C which was employed for all experiments.

The exchange reaction between p-IPPA and $Na^{131}I$ as in the above equation 1 is a simple homogeneous radioisotope exchange reaction and its kinetics will follow the exponential exchange law (20).

$$-\ln(1-F) = \frac{[A]+[B]}{[A].[B]} Rt$$

where F = fraction of exchange, [A] = the concentration of p-IPPA in mol L^{-1} (M), [B] = the concentration of $Na^{131}I$ (nca), R= isotope exchange rate, t= time in min.
The data in Fig. 2 were recalculated to obtain F (fraction of exchange) which is equal to :

$$F = X/X_\infty$$

where X and X_∞ are the radiochemical yields (% labelling) at time t and at equilibrium ($t=\infty$), respectively. A plot of Log (1-F) vs time (min.) gave a straight line, Fig.8.

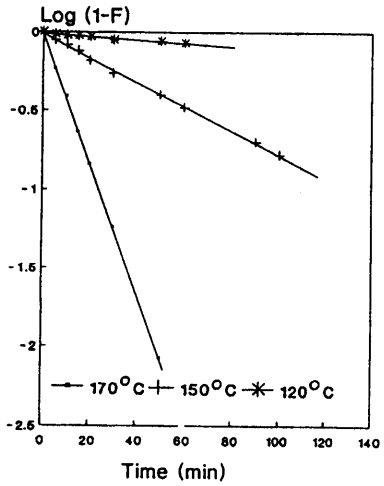


Fig. 8 : Variation of log (1-F) as a function of reaction time at different temperatures in dry state [3.7 MBq $Na^{131}I$ + 1mg p-IPPA +3mg benzoic acid].

It is clearly demonstrated that the exchange reaction between [$^{131}I^-$] iodide and p-IPPA follows the exponential exchange law; the straight line with a negative slope passes through the origin. In the exchange reaction , iodide (I^-) is unlikely to be oxidized to iodine (I_2); therefore it is possible that the exchange reaction is a nucleophilic reaction (21). By using the exponential exchange law , it is easy to conclude that the exchange reaction is a second order one

$$R = K [A] [B],$$ K= the specific rate constant

The specific rate constant (K) was calculated from the slope (p) of the plot of Log (1-F) against t, Fig. 8, according to the Mckay 's first -order exchange law describing homogeneous isotopic exchange processes (22).

$$-2.303 \text{ Log } (1-F) = pt$$

where p is the slope of the corresponding straight line .

$$K = \frac{2.303 \times p}{a}$$

where $a = [^{131}I^-] + [\text{p-IPPA}] \approx [\text{p-IPPA}] = 0.01126$ mol L^{-1}

NCA [$^{131}I^-$] iodide tracer was negligible considering that the radioactivity used in each run was only 10 µl, 3.7 MBq compared to the quantity of p-IPPA . The half time of exchange ($t_{1/2}$) corresponding to F = 0.5 is :

$$t_{1/2} = \frac{0.693}{K([A]+[B])} = \frac{0.693}{Ka}$$

The values of the slope (p) , K and $t_{1/2}$ at different temperatures are shown in table 4.

Table 4 : Variation of the slope (p) , specific rate constant (K) and half-time of exchange ($t_{1/2}$) with temperature .

Temperature (°C)	[p-IPPA] (M)	Slope ($min.^{-1}$)	K (M^{-1} $min.^{-1}$)	$t_{1/2}$ (min)
120	0.01126	7.139×10^{-4}	0.158	970.7
150	0.01126	8.01×10^{-3}	1.778	86.5
170	0.01126	0.041	9.102	16.9

It is clear that the specific rate constant K increases and $t_{1/2}$ decreases with temperature . Reaction rate constant, K value, can be obtained according to the Arrhenius equation:

$$K = Ae^{-\Delta E/RT}$$

where A= frequency factor, E= activation energy, R= gas constant, T=absolute temperature.
On plotting Log K vs. 1/T , Fig 9, the activation energy of this exchange reaction was calculated and found to be E = 33.54 K cal /mol.

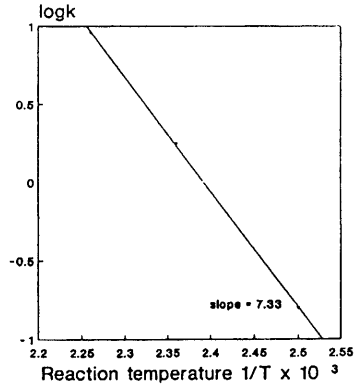


Fig. 9 : Relationship between reaction rate constant log k and reaction temperatures 1/T (T= absolute temperature) in dry state [3.7 MBq Na ^{131}I + 1mg p-IPPA + 3mg benzoic acid].

Employing pivalic acid as an exchange medium, it has been found that the rate of isotopic exchange was increased with the amount of pivalic acid added. This can be attributed mainly to the acidity of pivalic acid which facilitates the exchange reaction. Radioisotope exchange in the melt was found to be effective for the labelling of many iodoaryl compounds at

high temperature reaction conditions using acetamide (23), benzoic acid (13) and pivalic acid (15) as molten reaction media. Pivalic acid, with its low melting point (33°C) and weak acidity, appeared to be suitable for radioiodination of p-IPPA. Radioiodination of p-IPPA in pivalic acid and benzoic acid as well as acetamide resulted in the following relative labelling efficiencies: benzoic acid > pivalic acid >> acetamide, as shown in Fig. 10.

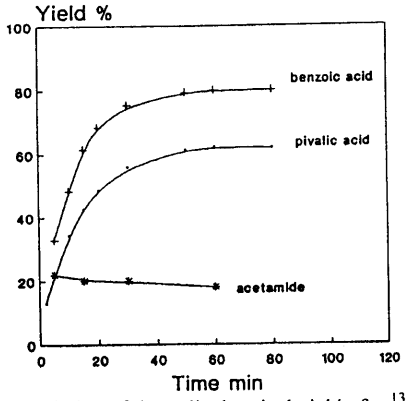


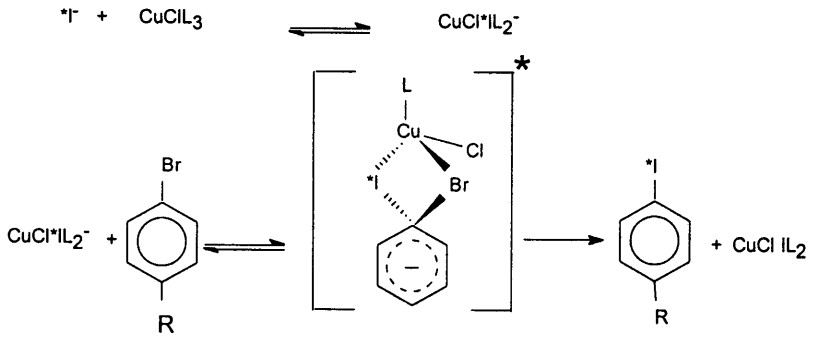
Fig. 10 : Variation of the radiochemical yield of p-^{131}IPPA as a function of various media [3.7 MBq $Na^{131}I$ + 1mg p-IPPA + Xmg different media]Temp. = 170°C.

On labelling of p-IPPA with ^{131}I in the dray state using ammonium acetate and a fixed amount of benzoic acid (3mg) at 120°C, the radiochemical yield of p-^{131}IPPA was increased from 41.74 to 60%. The increase of the radiochemical yield of p-^{131}IPPA may be attributed to the mildly acidic conditions provided by the thermal decomposition of ammonium acetate (24). There may be other iodinating species such as $NH_4{}^{131}I$ which may arise from the gradual increase of formation of $NH_4{}^+$ ions as a result of the decomposition of ammonium acetate to CH_3COO^- and $NH_4{}^+$. In this case the radioactivity will be obtained as $NH_4{}^{131}I$ which may be more reactive (25) than $Na^{131}I$.

Higher quantities of ammonium sulfate > 10 mg led to a decrease in the labelling yields which is probably due to the formation of large amounts of volatile iodine upon addition of ammonium sulfate to the substrate / radioiodide mixture (15). The observed decrease in the radiochemical yield is not in agreement with the results obtained earlier (14) on labelling of meta -iodobenzyl guanidine using ammonium sulfate (25mg) which gave a higher yield than 90 % and a negative effect when amounts of ammonium sulfate higher than 50 mg were used.

In order to elucidate the influence of benzoic acid on the radiochemical yield of p-^{131}IPPA, the iodine isotope exchange was performed in ethanol with 3 mg benzoic acid. Fig. 5 shows the result of this experiment. Fast incorporation of radioiodide occurred rapidly with a radiochemical yield of ≈ 80 % within 50 min, probably due to the solvent effect and hydroxide ion scavenger of benzoic acid (13). It is well known that the radioiodide solutions are usually admixed in 0.1 M NaOH which shows an undesirable effect on the radiochemical yield of p-^{131}IPP (13). Also, the exchange of iodine attached to the aromatic phenyl ring needs an acidic environment (26).

High radiochemical yield,≈77% of p-^{131}IPPA was achieved on labelling of p-IPPA with ^{131}I in presence of CuCl This high efficiency can also be attributed to the preferential coordination of [^{131}I] iodo-copper species with aromatic iodine atom prior to *ipso* substituon (16) according to the following reaction :



Hypothetical reaction mechanism for cuprous chloride - assisted NCA aromatic iododebromination.

A high isotopic exchange yield is achieved with the molar ratio 0.89, this corresponds to a cuprous chloride concentraion of about 10 mM . Above the CuCl / p-IPPA molar ratio of 0.89, the exchange yield decreased, which may reflect the formation of inorganic copper [^{131}I] iodide species which are unable to take part in the mechanism responsible for the exchange reaction (16). Lower concentrations of cuprous chloride led to a decrease in the labelling yield , probably due to insufficient interaction between copper species and aromatic substrate (16). A minimum amount of CuCl, required to initiate a reaction is about 50 μg (2.4 mM) for a 200 μl reaction volume. In the case of a zero molar ratio of CuCl/ p-IPPA, a very poor percent yield of the reaction was obtained.

conclusions

High radiochemical yield of p-^{131}IPPA (~80%) was obtained by isotopic exchange between p-IPPA and ^{131}I at mild temperatures for sixty minutes . This work has shown that cuprous chlorid-assisted nca aromatic iododebromination is useful for the rapid and regiospecific introduction of radioiodide onto aromatic rings . Cuprous chloride has the advantage of being, a comparatively innocuous reagent with low oxidation potential, when used in such low concentrations. Radioiodination of p-IPPA in pivalic acid and benzoic acid as well as acetamide resulted in the following relative labelling efficiency order :

benzoic acid > pivalic acid >> acetamide

Because of its rapidity, the method is appropriate for use with ^{123}I($t_{1/2}$=13.3h), currently the iodine nuclide of choice for scintigraphic imaging.

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