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Evaluation of the Reaction Parameters and Thermodynamics of the Iodide-Exchange Preparation of Radioiodinated 15-(p-iodophenyl) Pentadecanoic Acid by Both Solid Phase and Solution Methods

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SUMMARY

Two isotopic exchange procedures are described for the labelling of 15-(p-iodophenyl) pentadecanoic acid with radioiodine in the presence of ammonium sulphate and cuprous chloride as catalysts. The methods afforded regioselective and high specific activity products with radiochemical yields of 92% and 85% within relatively short reaction times. High pressure liquid chromatographic separation resulted in high radiochemically pure product suitable for medical application. Kinetic studies revealed second order iodine-iodine exchange reactions with an activation energy of 13.7 Kcal /mole for the solid state radioiodination with ammonium sulphate and 15.3 Kcal/mole for the copper catalyzed exchange reaction.

Key words: Radioiodination, fatty acids, cuprous chloride, ammonium sulphate, activation energy.

INTRODUCTION

Radioiodinated fatty acids are suitable radiopharmaceuticals for myocardial scintigraphy in some heart disorders. Since aliphatic fatty acids undergo deiodination in vivo, Machulla (1) stabilized the carbon-iodine bond by replacing the alkyl carbon-iodine bond by an aryl carbon-iodine bond. Thus, 15-(p-iodophenyl) pentadecanoic acid, IPPA, was proposed and synthesized as an alternative concept for radioiodinated fatty acids (2). The important features that make the phenyl fatty acids more attractive than their aliphatic analogues, are their better chemical stability and their more favourable catabolism. Radioiodinated aliphatic fatty acids are finally catabolized to radioiodine, whereas radioiodinated p-IPPA is degraded via B-oxidation to p[*I] iodobenzoic acid which is rapidly excreted from the body as [*I] iodohippuric acid (3) and thus lowering undesirable background radiation. Enzymatic deiodination is also reduced in compounds where iodine is located in the para position with respect to the side chain. These properties make iodine labelled 15-(p-iodophenyl) pentadecanoic acid an interesting radiopharmaceutical for myocardial imaging (4,5).

The synthesis of radioiodinated p-IPPA can be achieved by electrophilic aromatic substitution of the para hydrogen on the phenyl group of 15-phenyl pentadecanoic acid. This technique is not regiospecific and extensive HPLC purification is required to separate the *ortho* (29%) and *para* (71%) isomers (6). Since only the *para* isomer is suitable for clinical application, a reliable method of introducing radioiodine label into the *para* position was desirable. Eisenhut (7) first demonstrated the applicability of isotope exchange technique for radioiodinating IPPA in a solid

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phase using benzoic acid. Solid phase exchange using ammonium sulphate (8) and exchange in acetic acid (9) have been also reported. The isotopic exchange method has been improved considerably when CuCl was used in order to obtain radioiodinated IPPA in high specific activities for routine clinical application (10). Verbruggen described a procedure for labelling IPPA with ^{123}I using Sn in an acidic medium (11). Labelling, characterization and biodistribution of [^{131}I] IPPA has been performed (12). Comparison between 2 kinds of radioiodine labelling methods of 15-(p-[^{125}I] iodophenyl) pentadecanoic acid has also been carried out (13). P-IPPA has been synthesized and labelled by the exchange method (14) and a simple and efficient method for its preparation has been reported (15). A procedure for labelling p-IPPA with ^{131}I by isotopic exchange in ethanol using benzoic acid was described (16).

In this work, radioiodinated p-IPPA has been prepared by the isotopic exchange technique both in the aqueous and the solid states. The reaction parameters and the thermodynamics of the exchange reactions have been evaluated.

EXPERIMENTAL

Materials:

^{131}I used in this study is a carrier-free and reductant-free solution of $Na^{131}I$ in 0.1N NaOH locally produced in the Egyptian reactor by dry distillation from irradiated TeO_2 (act. conc.=50 mCi/ml).

15-(p-iodophenyl) pentadecanoic acid was purchased from Cambrian chemicals (USA), Cuprous chloride (Merck), ammonium sulphate (Fischer), sodium metabisulphite (BDH) were used without further purification.

Solvents for high pressure liquid chromatography were high purity grade.

All other solvents and reagents used in this work were of analytical grade.

Solid State Labelling:

In a glass ampoule, 500 μg of 15-(p-iodophenyl) pentadecanoic acid and the desired amount of ammonium sulphate were dissolved in ethanol followed by the addition of 10 μl I-131 (100 μCi) in 0.1N NaOH. The solvent was evaporated to dryness and the glass ampoule sealed. The vial was heated to the desired temperature for different periods of time in a thermostatically-controlled oil bath after which the vial was cooled and the reaction mixture dissolved in ethanol before chromatographic analysis.

Aqueous State Labelling:

Aqueous solutions of cuprous chloride, sodium metabisulphite and 10 μl (100μCi) I-131 in 0.1N NaOH were added to a V-shaped reaction vial. The solution was evaporated to dryness in a water bath, then 500 μg of 15(p-iodophenyl) pentadecanoic acid in 200 μl glacial acetic acid were added to the residue. The tube was sealed and the reaction mixture heated in an oil bath at the selected temperature. The tube was dipped 1cm into the oil bath to allow the acetic acid to reflux on the wall of the tube. The reaction was allowed to proceed for a chosen interval of time after which the contents of the tube were subjected to chromatographic analysis.

Radiochemical yields of the exchange reactions were determined by thin layer chromatography, TLC, on Merck Silica Gel-60 aluminium backed plates using diethyl ether as the developing solvent. The R_f for the iodinated p-IPPA is 0.9 while the unreacted iodide remains near the origin. Separation of the labelled product was also achieved by means of high pressure liquid chromatography on reverse phase RP-18 column (250x4mm), Lischrosorb, Merck, eluted with methanol : water : acetic acid (95:3:2, v/v/v) at a flow rate of 1 ml/min. The retention time of radioiodinated IPPA was 19 min, and that of radioiode was 2 min. The radiochemical yield is determined as

the ratio of the radioactivity of the labelled product to the total radioactivity on the radiochromatogram or injected into the column. Radiochemical yields are expressed as the mean value of two experiments.

RESULTS AND DISCUSSION

The need for an acidic environment for the exchange of iodine attached to the aromatic ring has been demonstrated (7,17). The sodium hydroxide present in commercially available radioiodide solutions has a negative effect on the yield of the exchange reactions (7,10). Neutralization of the sodium hydroxide and acidification of the reaction medium can be achieved by adding a sufficient quantity of the fatty acid to be exchanged which limits the specific activity of the product. Thus a low fatty acid concentration is necessary due to both its limited solubility in human serum albumin and to obtain high specific activity radioiodinated IPPA. Another approach is to add an alternative source of protons to the reaction mixture. Benzoic acid serves as a solvent and hydroxide ion scavenger by which high exchange yield of radioiodinated IPPA has been obtained (7), but it requires additional purification steps prior to human use. Ammonium sulphate is an excellent alternative, it provides an acidic reaction medium and since both ammonium and sulphate ions are compatible with human use, it requires no removal prior to injection. A high radiochemical yield of radioiodinated p-IPPA has been reported when using ammonium sulphate (8).

Fig.(1) shows the effect of increasing the amount of ammonium sulphate on the radiochemical yield of 15-(p-[^{131}I] iodophenyl) pentadecanoic acid at 170°C. Data indicate that increasing the amount of ammonium sulphate increases the radiochemical yield which was attributed to the increase in the acidity of the medium due to the thermal decomposition of $(NH_4)_2SO_4$ and the loss of ammonia. The increase in the acidity of the medium facilitates the exchange reaction. A maximum radiochemical yield was obtained with 10 mg ammonium sulphate.

Fig.(2) shows the dependence of the radiochemical yield of the exchange reaction between radioiodide and p-IPPA on the reaction time at different temperatures. The reaction was performed with 500 µg p-IPPA, 10 mg $(NH_4)_2SO_4$ and 0.001 m.mole NaOH. The presence of excess $(NH_4)_2SO_4$ with respect to NaOH prevents its influence. It can be observed that above the melting point of p-IPPA at 130°C, the rate of reaction was rather slow by comparison to the exchange rate at 170°C which indicates that temperature has a great effect on the reaction rate. Since p-IPPA is stable up to 190°C, elevated temperatures were used to accelerate the exchange rate and decrease the reaction time.

Catalysts are also used to decrease the reaction time and improve the labelling yield in isotopic exchange reactions especially in radioiodination reactions with the short-lived ^{123}I-isotope. Copper salts have proven to be useful for the catalysis of isotopic exchange of radiobromide and radioiodide into aromatic sings (18-20). In our previous investigation on the copper-catalyzed radioiodination of 3-iodotyrosine and 4-iodophenylalanine (21), it was found that the use of a reducing agent in the presence of CuCl improved the yield and decreased the amount of side products. The reducing agent prevents the oxidation of Cu(I) and avoids the formation of I_2 and the loss of activity. The use of sodium metabisulphite has solved the practical limitations associated with the use of ascorbic acid (yellow coloration) or stannous chloride (white precipitate of hydrolyzed tin).

Fig.(3) shows the effect of increasing the molar ratio CuCl /p-IPPA on the radiochemical yield of 15-(p-[^{131}I]iodophenyl) pentadecanoic acid at 170°C in the presence of a constant amount of 1 mg sodium metabisulphite as reducing agent in acetic acid. The maximum yield is obtained at a molar ratio CuCl /p-IPPA of 0.91. The relation of Cu(I) concentration and radiochemical yield can be interpreted in terms of the 2-step mechanism proposed by Moerlein (22) which involves the formation of

transient *in-situ* organo-copper complex which favours the exchange of radioiodine with the inactive iodine in the iodocompound. Excessive as well as insufficient concentrations of cuprous chloride are inadequate to obtain optimum radioiodination yields.

The effect of temperature on the radiochemical yield of 15-(p-[^{131}I] iodophenyl) pentadecanoic acid was studied as a function of time using 500 µg p-IPPA, 100 µg CuCl (CuCl /p-IPPA molar ratio = 0.91) and 1 mg $Na_2S_2O_5$, Fig.(4). Data indicate that the yield increases with increasing temperature which has a great effect on the exchange reaction rate. Without catalyst the rate of the reaction was slow and a low yield of 20% was obtained. This corresponds to a mechanism involving very high activation energy. The catalyst lowers the activation energy by providing an alternative mechanism thereby permitting the reaction to proceed at an appreciable rate (23).

The kinetics of the exchange reaction will follow the exponential exchange law:

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

$$F \text{ is the fraction of exchange} = \frac{\text{Yield } (t)}{\text{Yield } (\infty)}$$

[A] is the concentration of p-IPPA , [B] is the concentration of Na ^{131}I
(t) is the time , V is the isotope exchange rate=K [A] [B]

The data are recalculated to the exchange fraction F and ln (1-F) is plotted as a function of time(t) in minutes, Figs.(5 and 6). A straight line passing through the origin is obtained for all reaction temperatures. This suggests that the exchange reaction is a second order iodine-iodine exchange. In the radioiodination of N,N,N`-trimethyl-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propanediamine(HIPDM), results proved that the reaction is also a second order exchange reaction (24).

The rate of reaction at each temperature is calculated from the slope of the plot of ln (1-F) against time according to the following equation

$$\ln (1-F) = - kat$$

where k is the reaction rate constant
t is the time
a = [p-IPPA] + [Na ^{131}I]

The Arrhenius equation can be applied by plotting ln k against $^1/_T$, Figs.(7,8) according to the following equation

$$k = A\, e^{-E/RT}$$

$$\ln k = \ln A - \frac{E}{RT}$$

where A is the frequency , R is the gas constant
T is the absolute temperature , E is the activation energy

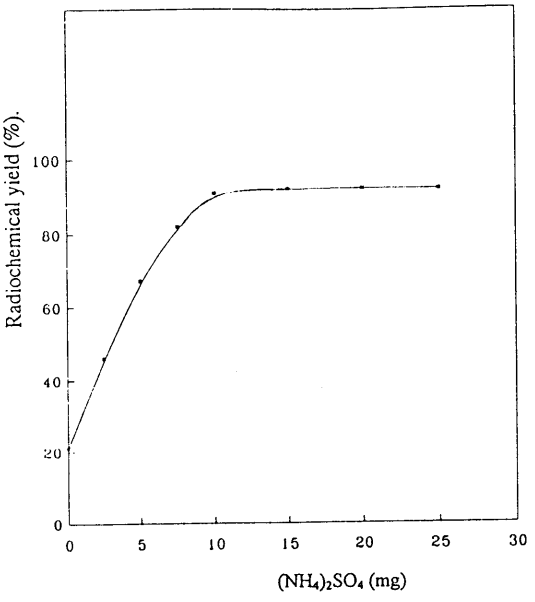

Fig.(1): Variation of the radiochemical yield of [^{131}I] IPPA with the amount of ammonium sulphate.
(500 µg p-IPPA + x mg $(NH_4)_2SO_4$ + 100 µCi $Na^{131}I$)

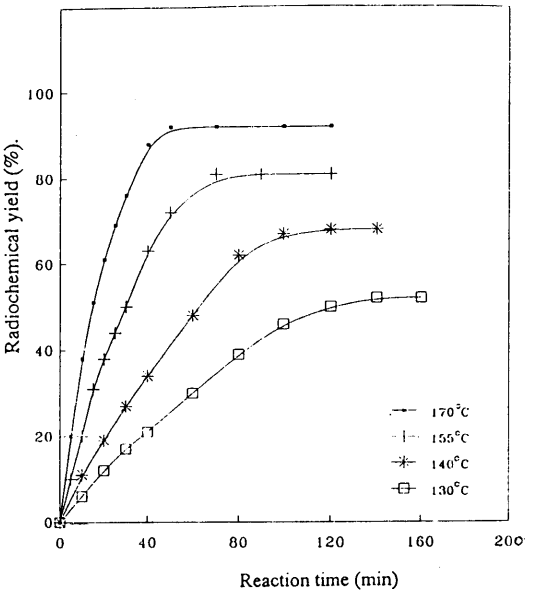

Fig.(2): Variation of the radiochemical yield of [^{131}I] IPPA with time at different temperatures
(500 µg p-IPPA + 10 mg $(NH_4)_2SO_4$ + 100 µCi $Na^{131}I$)

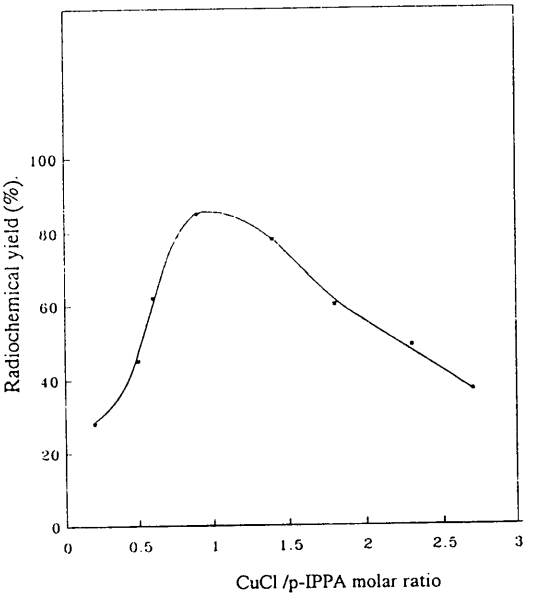

Fig.(3): Relation between radiochemical yield of p-[^{131}I] IPPA and molar ratio CuCl /p-IPPA.

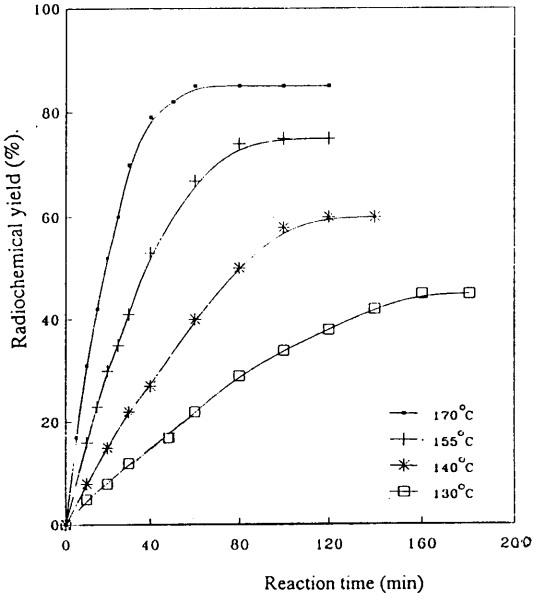

Fig.(4): Variation of the radiochemical yield of [^{131}I] IPPA with time at different temperatures
(500 µg p-IPPA +100 µg CuCl +1 mg $Na_2S_2O_5$ + 100 µCi Na ^{131}I)

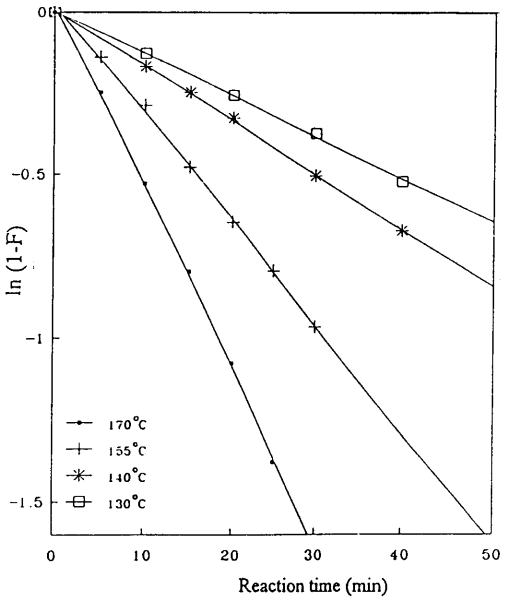

Fig.(5): Variation of ln (1-F) with time at different temperatures. (500 µg p-IPPA + 10 mg $(NH_4)_2SO_4$ + 100 µCi $Na^{131}I$)

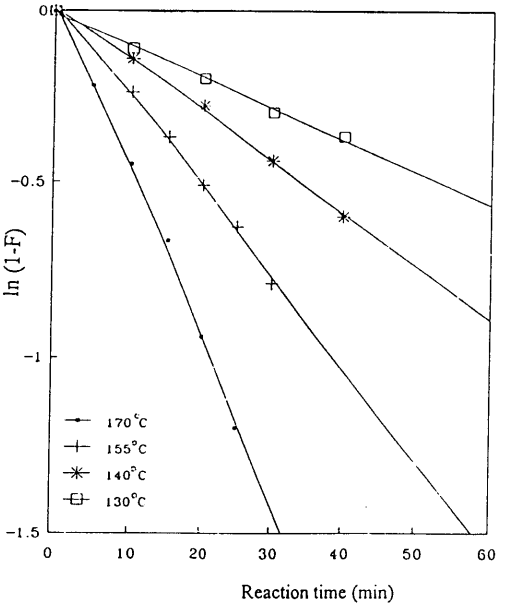

Fig.(6): Variation of ln (1-F) with time at different temperatures (500 µg p-IPPA +100 µg CuCl +1 mg $Na_2S_2O_5$ + 100 µCi Na ^{131}I)

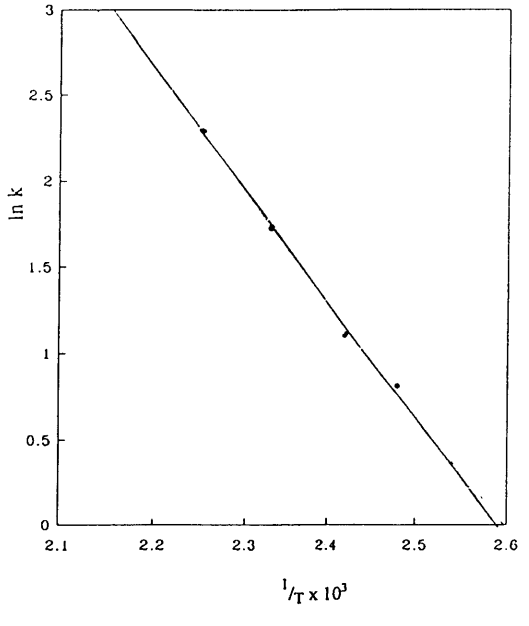

Fig.(7): Relation between ln K and $^1/_T$ (500 µg p-IPPA + 10 mg $(NH_4)_2SO_4$ + 100 µCi $Na^{131}I$)

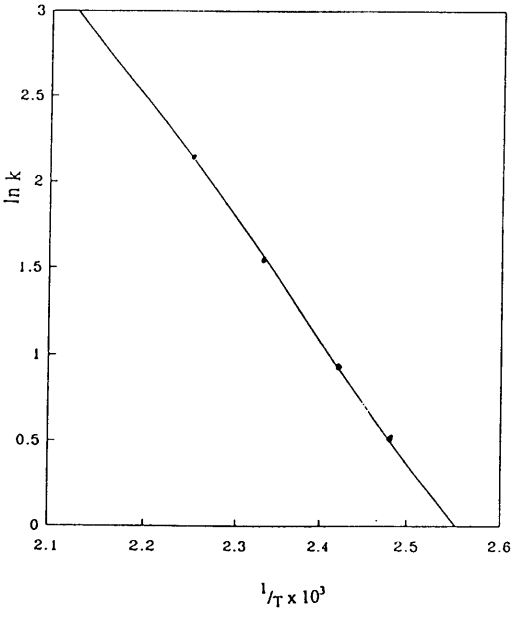

Fig.(8): Relation between ln K and $^1/_T$ (500 µg p-IPPA +100 µg CuCl +1 mg $Na_2S_2O_5$ + 100 µCi Na ^{131}I)

Examination of the data indicates that in the presence of the CuCl catalyst, the reaction time was 60 ± 1 min., while in the presence of ammonium sulphate, the reaction time decreased to 50 ± 1 min at 170°C indicating a faster reaction at a given temperature. Thus a lower activation energy of 13.7 Kcal/mole is obtained in the isotopic exchange reaction using ammonium sulphate while an activation energy of 15.3 Kcal/mole is obtained for the copper catalyzed exchange reaction between radioiodide and p-IPPA. In addition, the exchange in the presence of ammonium sulphate employs no metals or solvents incompatible with human use and purification can be performed by dissolving the reaction mixture in aqueous solution or ethanol and passing it through an anion exchange column to remove free iodide. High pressure liquid chromatographic separation can also be performed on a RP-18 column eluted with a solvent mixture of methanol:water:acetic acid (95:3:2). The eluted fractions containing the labelled fatty acid are collected and evaporated under vacuum, then dissolved in 300 µl ethanol and added dropwise to 2 ml 5% HSA in an ultrasonic water bath. Finally the solution is sterilized by filtration to be ready for administration. Using high specific activity ^{123}I in the labelling procedure, p-[^{123}I] iodophenyl) pentadecanoic acid can be prepared and used for myocardial imaging by means of single photon emission computed tomography (SPECT).

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