Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIX, No. 11

COPPER CATALYZED RADIOIODINATION OF 3-IODOTYROSINE AND 4-IODOPHENYL ALANINE.

FARAH K. and FAROUK N.

Labelled Compounds Department, Hot Laboratories Centre,
Atomic Energy Authority, P.O. Code 13759, Cairo, Egypt.

SUMMARY

The factors affecting the yield of the copper catalyzed labelling of 3-iodotyrosine and 4-iodophenyl alanine such as pH of the medium, catalyst and substrate concentrations and solvent effects are described. The reaction is assumed to take place via an intermediate organo-copper complex which favours the exchange between radioiodine and the inactive iodine in the iodocompound. The reducing agents ascorbic acid, stannous chloride or sodium metabisulphite are added to the cuprous chloride catalyzed reaction to prevent the oxidation of Cu (I) to Cu (II) in order to decrease side products formation and improve the labelling yield. Kinetics indicated a second order reaction with an activation energy of 9.6 Kcal / mole for 3-iodotyrosine and 12.2 Kcal / mole for 4- iodophenyl alanine. The stronger 4-iodophenyl C-I bond favours para-iodination in the preparation of radioiodinated monoclonal antibodies.

Keywords : Isotopic exchange , 3-iodotyrosine, 4-iodophenyl alanine, cuprous chloride, activation energy.

INTRODUCTION.

Radioiodinated compounds can be prepared either by electrophilic radioiodination reactions or by nucleophilic exchange reactions. Activated compounds containing electron accepting groups exhibit strong reactivity under nucleophilic substitution requiring only a short reaction time. Non-activated aryl iodides require higher temperatures to effect the exchange. Accordingly high boiling point solvents have been utilized with some success. Compounds which do not exchange well with radioiodide in solvents can give better yields when the iodocompound is melted with radioiodide in acetamide, formamide, benzoic acid or ammonium sulphate.

Catalysts are also used to improve the radiochemical yield and decrease the reaction time in isotopic exchange reactions especially in radioiodination reactions with the short lived ^{123}I isotope. Copper compounds have been

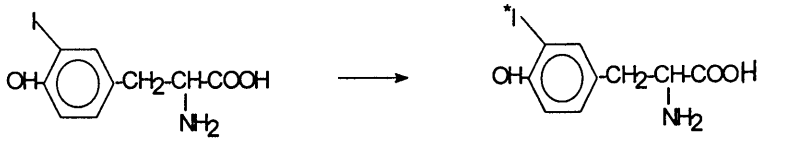
CCC 0362–4803/97/011915–12$17.50

Received 12 May 1997
Revised 28 July 1997

successfully employed for promoting nucleophilic substitution reactions in non-activated aromatic compounds which lack electron withdrawing substituents (1). Copper (I) salts have proven to be useful for the catalysis of isotopic exchange reactions in the synthesis of radioiodinated N-isopropyl p-iodoamphetamine (2), m-iodobenzyl guanidine (3,4), o- iodohippuric acid (5) and the receptor binding ligands spiperone (6) and ketanserine (7).

Phenyl alanine, tyrosine and its hydroxylated form, Dopa, are biologically important molecules. They can be regarded as precursors for adrenomedullary hormones and melanin. To utilize these compounds in nuclear medicine, they were labelled with γ-emitting radioisotopes. The various iodophenyl alanine isomers have been used for pancreatic imaging studies (8) and tumour localization. Some aromatic amino acids were proven to be potent tracers for studying brain metabolism in vivo ; 6-[^{18}F] Fluorodopa for probing the dopamine pool in the brain (9), 4-[^{18}F] Fluorophenyl alanine for measuring cerebral protein synthesis (10) and L-[^{11}C] tyrosine for the determination of protein synthesis rate in tumor tissue and brain (11). N-acyl derivatives of p- iodophenyl alanine labelled with radioiodine showed good affinity for the pancreas (12). P-iodophenyl alanine has been labelled with ^{123}I by isotopic exchange in the melt (13). The catalytic effect of copper on the radioiodination of p-iodophenyl alanine has been investigated (14). 6-[^{18}F] and 4-[^{18}F] fluoro- L-m-tyrosine have been synthesized via regioselective radiofluorodestannylation (15).

To improve the radiochemical yield and decrease the reaction time for the exchange reaction of the non-activated 3-iodotyrosine and 4-iodophenyl alanine, cuprous chloride is used as a catalyst in this work.



3-iodotyrosine

4-iodophenyl alanine

EXPERIMENTAL

Materials

3-iodotyrosine (Sigma Chem.Co), 4-iodophenyl alanine (Sigma Chem. Co), cuprous chloride (Merck), ascorbic acid (BDH), stannous chloride (Aldrich Chem. Co) and sodium metabisulphite (BDH) are used without further purification. ^{131}I is a carrier free and reductant free $Na^{131}I$ in diluted NaOH. Solvents for high pressure liquid chromatography are high purity grade. All other solvents and reagents used in the work are of analytical grade.

Labelling technique

The radioiodination reactions are carried out in tightly sealed glass reaction vials heated to selected temperature. The substrate is added to 500 µl aqueous HCl, followed by the addition of CuCl and 5 µl of radioactive sodium iodide (50 µCi). The reaction is allowed to proceed for a chosen interval of time after which the products were then analyzed on whatman No.3 paper chromatography using n-butanol : acetic acid : water (4 :1 :1) as developing solvent. Iodide remains near the origin while 3-[^{131}I] iodotyrosine and 4-[^{131}I] iodophenyl alanine move with R_f = 0.5 and 0.7 respectively. Separation and purification of the labelled products were also achieved by means of high pressure liquid chromatography on RP-18 column, 250 x 4 mm , Lichrosorb, Merck using 0.02M sodium acetate : ethanol (9:1) for 3-iodotyrosine and acetonitrile : water (1:1) for 4-iodophenyl alanine as eluting solvents. The radiochemical yield is calculated as the ratio of the radioactivity of the labelled compound to the total radioactivity.

RESULTS AND DISCUSSION.

Effect of pH

The exchange reaction is studied at various pHs ranging from 1 to 10. Reaction temperature was held at 100°C and samples were analyzed after a reaction time of 100 min. when the reaction pH was below 7, the exchange reaction was found to proceed smoothly reaching a maximum labelling yield at pH 3. Thus the reaction prefers acidic pHs; the optimum pH for the exchange reaction of 3-iodotyrosine and 4-iodophenyl alanine is around 3. All the aqueous exchange reactions are thus carried out at pH 3 adjusted with HCl. It is known from other exchange reactions i.e. from the preparation of radioiodinated o-iodohippuric acid that the exchange of iodine attached to the aromatic phenyl ring needs an acidic environment (16) .

Effect of substrate conc.

The dependence of the radiochemical yield of 3-[^{131}I] iodotyrosine and 4-[^{131}I] iodophenyl alanine on the amount of substrate using a constant

amount of cuprous chloride as catalyst was investigated. The results show that there is a small increase in the yield which becomes constant with further increase in the amount of substrate over the investigated range from 0.2 to 2 mg. A constant amount of 1mg substrate was used throughout the work.

Effect of catalyst conc.

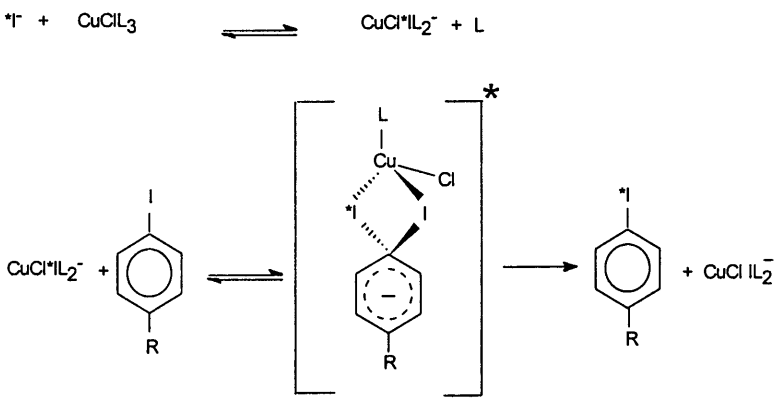
The influence of copper (1) chloride concentration on the radiochemical yield of 3-[^{131}I]iodotyrosine and 4-[^{131}I]iodophenyl alanine was studied to determine the optimum concentration of catalyst which is required to maximize radiochemical yield and minimize damage to the substrate molecules and side products formed by the copper catalyzed side reactions. The relation between the amount of cuprous chloride and the radiochemical yield is illustrated in Fig (1). The maximum value for the radiochemical yield is obtained at $2x10^{-2}$ M CuCl. Although Cu (I) exhibits a catalytic effect on the reaction , yet its amount is important. The optimum radiochemical yield occurs only within a certain range of concentration. Excessive as well as insufficient concentrations of cuprous chloride are inadequate to obtain optimum radioiodination yields. The relation between the Cu (I) concentration and the radioiodination yield can be interpreted in terms of the 2-step mechanism proposed by Moerlein (17) which involves the formation of transient in-situ organo-copper complex which favours the exchange of the radioiodine with the inactive iodine in the iodocompound.

Low concentrations of copper (1) chloride leads to a decrease in the labelling yield due to insufficient interaction between cuprous chloride and radioiodide to form the reactive radioiodinating species; this [*I] copper complex coordinates with the aromatic substrate where carbon-iodine bond as well as copper-iodine coordinations occur. At high concentrations of copper (I) chloride, the labelling yield decreases due to disproportionation of the copper (I) ions as the molarity of cuprous chloride is increased.

$$2\,Cu^{+} \;\leftrightarrows\; Cu^{o} + Cu^{++}$$

so the concentration of reactive radioiodinating species is diminished with the formation of inorganic copper iodide ($Cu^{131}I$) species which are unable to take part in the radioiodination reaction.

The following equations represent the hypothetical reaction mechanism of cuprous chloride assissed aromatic radioiodination .



The use of Cu (I) salts (CuCl) results in somewhat low labelling yields and the formation of side products. This can be explained by the fact that Cu (I) is not stable in solution resulting in the formation of Cu (I I) which reacts with $*I^-$ to form $Cu*I + *I_2$ which are reagents propagating electrophilic reactions (2). Cupric salts are generally assumed to be effective agents for electrophilic (18) but not for nucleophilic substitution reactions. The labelling of 3-iodotyrosine and 4-iodophenyl alanine with cuprous salt (CuCl) as a catalyst in the presence of ascorbic acid, stannous chloride and sodium metabisulphite as reducing agents has been investigated. It was found that the addition of excess ascorbic acid or stannous chloride (10mg) to the reaction mixture containing 1mg CuCl improves the labelling yields and decreases the amount of side products (table 1). It seems that the role of the reducing agent is to prevent the oxidation of Cu (I) to Cu (II) so that Cu (I) remains during the course of the reaction without the formation of radiolabelled or cold side products (19). The use of a reducing agent also avoids the formation of $^{131}I_2$ resulting from the oxidation of $^{131}I^-$ by Cu(II) and the loss of activity due to the volatility of $^{131}I_2$ (2). The problem associated with the use of ascorbic acid is the yellow coloured solutions resulting from the oxidation of ascorbic acid in the presence of water, Cu ions and heat which may inhibit the complete reduction of copper (20). The use of $SnCl_2.2H_2O$ as reducing agent leads to a white precipitate which is attributed to the formation of hydrolyzed forms of tin Sn $(OH)^+$, Sn_2 $(OH)_2^{2+}$ or Sn_3 $(OH)_4^{2+}$ (21). The use of sodium metabisulphite $Na_2S_2O_5$ as reducing agent has solved these practical limitations. Sodium metabisulphite is used to stop radioiodination reactions because it is able to reduce iodine species to iodide form. When 10 mg of sodium metabisulphite are added to the reaction mixture, a high yield is obtained with no decoloration or white precipitate formation in the solution. Neves et al (4) has suggested the use of sodium metabisulphite, a mild reducing agent able to generate Cu (I) in situ and to keep iodine in a low oxidation state in the labelling of m-iodobenzyl guanidine using cupric sulphate $CuSO_4.5H_2O$ as catalyst.

Table (1)
Effect of the Addition of some Reducing Agents to the copper Catalyzed Exchange of 3-iodotyrosine and 4-iodophenyl alanine

Catalyst	Yield, % 3-iodotyrosine	4-iodophenyl alanine
CuCl	72	68
CuCl + Asc. Acid	78	72
CuCl + $SnCl_2.2H_2O$	82	75
CuCl + $Na_2S_2O_5$	86	79

Effect of temperature

3-[^{131}I] iodotyrosine and 4-[^{131}I] iodophenyl alanine are prepared by an exchange reaction between radioiodine and the inactive iodine in the iodo-compound in which iodine is bound to an aromatic carbon atom. The reaction proceeds by nucleophilic substitution of iodine atom via an intermediate where both radioactive and non-radioactive iodine atoms are symmetrically bound to the same carbon atom. The velocity of the reaction depends on the rupture of the C-I bond which is dependent on the temperature (22).

In isotopic exchange reactions, catalysts are used to improve the radio-chemical yield and decrease the reaction time, especially in compounds which are not activated to nucleophilic substitution. A catalyst is a substance which influences the rate of a reaction. As the rate of reaction is governed by the activation energy (heat of formation of an intermediate compound), the reactions which are extremely slow in the absence of a catalyst corresponds to the mechanism involving very high activation energy, and the catalyst lowers the activation energy by providing an alternative mechanism, thereby letting the reaction proceeds at an appreciable rate (23).

The effect of temperature on the radiochemical yield of 3-[^{131}I] iodo-tyrosine and 4-[^{131}I] iodophenyl alanine in the presence of Cu (I) Cl as catalyst was studied as a function of time and the results are shown in Figs (2 & 5). Data indicate that the yield increases with increase of temperature and there is a significant effect of temperature on the exchange rate.

In aqueous solution, the exchange reaction between radioiodine and the iodoaromatic compound is a homogeneous exchange reaction and its kinetics will follow the exponential exchange law :

$$-\mathrm{Ln}\,(1-F) = \frac{[A]+[B]}{[A]\,.\,[B]} \times V\,t$$

V is the isotope exchange rate = k [A] . [B]

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

[A] is the concentration of Na^{131}I

[B] is the concentration of substrate, (t) is the time.

The data is recalculated to the exchange fraction F and ln (1 - F) is plotted as a function of time (t) in minutes. The results given in Figs (3 & 6) clearly demonstrate that these copper catalyzed exchange reactions follow the exponential exchange law; a straight line passing through the origin is obtained for all reaction temperatures studied. This strongly suggests that the mechanism of this exchange reaction is a second order iodine-iodine exchange reaction. In the radioiodination of HIPDM and the radiobromination of 4-BrAP results proved that the exchange reaction is second order (24, 25).

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

$$\text{Ln}\,(1 - F) = -k\,a\,t$$

where k is the reaction rate constant.

t is the time

a = [Na I] + [subst]

Arrhenius equation can be applied by measuring the slope of the plot of ln k against $\frac{1}{T}$, Figs (Figs 4 & 7) according to the following equation

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

$$\text{Ln}\,k = \text{Ln}\,A - \frac{E}{RT}$$

Where A is the frequency,

R is the gas constant.

T is the absolute temperature.

E is the activation energy.

From the above data, it can be observed that without catalyst the rate of the reaction is slow and a very low labelling yield is obtained at 100 °C. This means that the exchange reaction needs high activation energy. The I -aryl bond strenght has been reported to be about 60 Kcal / mole for iodobenzene but the hydroxyl substituent reduces it to 54 Kcal /mole (26). In the presence of CuCl catalyst, the activation energy is calculated to be 9.6 Kcal / mole for 3-iodotyrosine (3-iodo, 4-hydroxyphenyl alanine) and 12.2 Kcal / mole for 4-iodophenyl alanine. Thus the function of Cu (I) is to decrease the activation energy which leads to a faster reaction at a given temperature.

Examination of the data shows that the 4-iodophenyl C – I bond is stronger than the 3-iodo 4-hydroxyphenyl bond where the hydroxyl substituent reduces the I-aryl bond strength. The most serious problem in the preparation of halogenated monoclonal antibodies (MAbs) is the deiodination secondary to catabolism of the radioiodinated antibodies. Thus enzymatic deiodination will be reduced in preparations where iodine is located in the para position with respect to the side chain. The three factors namely, bond strenght, susceptibility to enzymatic deiodination and pharmacokinetic fate of degradation products favour para-iodination in the preparation of

radio-iodinated monoclonal antibodies and the preparation of 4-[^{123}I / ^{131}I] iodo-phenyl alanine and 4-[^{18}F] fluorophenyl alanine used in nuclear medicine imaging.

Solvent effect

The CuCl catalyzed radioiodination of 3-iodotyrosine and 4-iodophenyl alanine in several organic media such as ethanol, methyl ethyl ketone, dimethyl sulphoxide, dimethyl formamide and acetonitrile has been investigated at 80°C (table 2) The labelling yields in the different solvents were of the order :

$$C_2H_5OH > MEK > DMF > CH_3CN > DMSO$$

The effect of solvents was not only associated with the dielectric constant or the donor number but determined by specific interactions possibly due to the nature of the transient state. Increasing the temperature of the exchange reaction to 140°C in the high boiling solvents, dimethyl formamide and dimethyl sulphoxide leads to an increase in the labelling yield to 48 & 43 in DMSO and 56 & 49 in DMF for 3-iodotyrosine and 4-iodophenyl alanine respectively.

Table (2)
Effect of Solvents on the Copper Catalyzed Exchange
of 3-iodotyrosine and 4-iodophenyl alanine

Solvent	Yield, % 3-iodotyrosine	4-iodophenyl alanine
C_2H_5OH	54	48
MEK	48	44
DMF	40	33
CH_3CN	35	29
DMSO	30	26

Optimum condition for labelling 4-iodophenyl alanine.

Fig (8) shows a radiochromatogram for the iodination of 4-iodophenyl alanine after high pressure liquid chromatographic separation on RP-18 column at the optimum condition (1mg substrate + 1mg CuCl + 10mg $Na_2S_2O_5$) at 100 °C. Using acetonitrile : water (1 :1) as eluting solvent at a flow rate of 0.5 ml / min, 4-iodophenyl alanine is eluted at a retention time of 10 min. The fractions were collected separately and the radioactivity measured by a γ-counter. The fractions containing the labelled compound are pooled together and evaporated to dryness and the residue is dissolved in physiological saline. Using ^{123}I in the labelling synthesis, 4-[^{123}I] iodophenyl alanine is prepared (specific activity 10 mCi / mg). After sterifiltration, the radiopharmaceutical is suitable for nuclear medicine imaging.

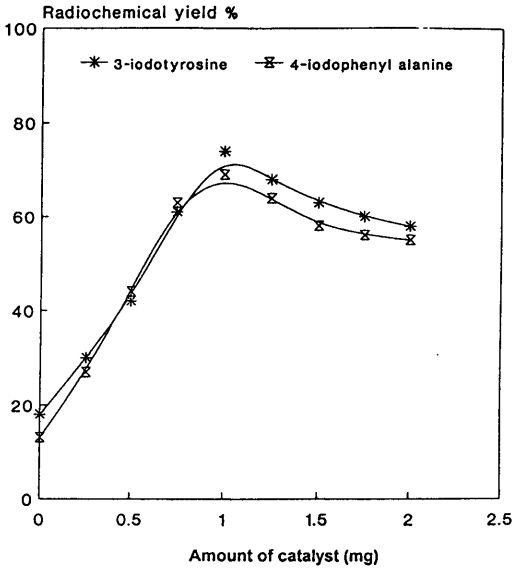


Fig (1) : Variation of the radiochemical yield of 3-[^{131}I] iodotyrosine or 4-[^{131}I] iodophenyl alanine with the amount of catalyst [1 mg substrate + x mg CuCl + 5µl Na^{131}I in 0.5 ml HCl (pH 3)] Temp. = 100°C Reaction time = 100 min.

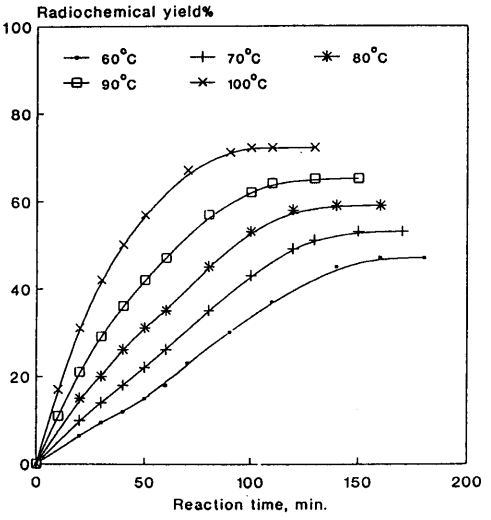


Fig (2) : Variation of the radiochemical yield of L-3-[^{131}I] iodotyrosine with time at different temperatures [1mg substrate +1 mg CuCl + 5µl Na^{131}I in 0.5 ml HCl (pH 3)]

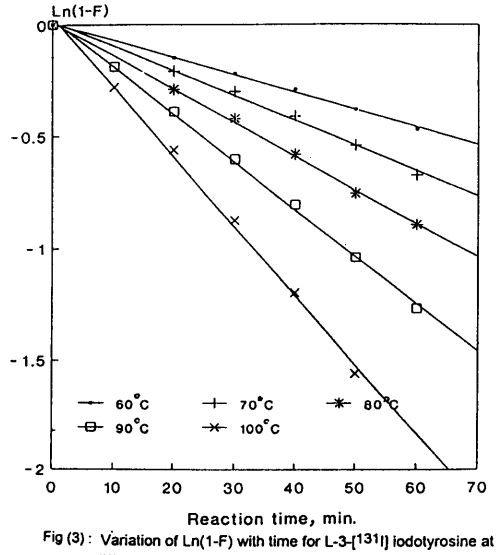


Fig (3) : Variation of Ln(1-F) with time for L-3-[^{131}I] iodotyrosine at different temperatures [1mg 3-iodotyrosine + 1mg CuCl+ 5µl Na^{131}I in 0.5 ml HCl (pH 3)]

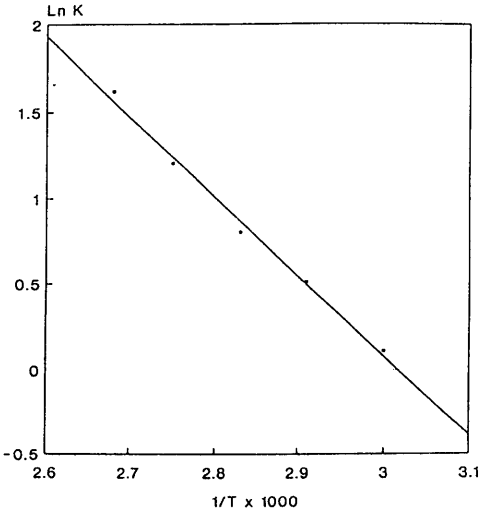


Fig (4) : Relation between Ln K and 1/T for 3- [^{131}I]iodotyrosine

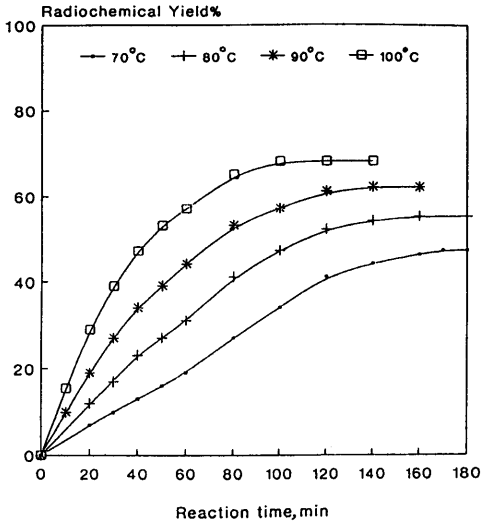


Fig (5): Variation of the radiochemical yield of 4-[^{131}I] iodophenyl alanine with time at different temperatures [1mg substrate + 1mg CuCl + 5µl Na^{131}I in 0.5 ml HCl (pH 3)]

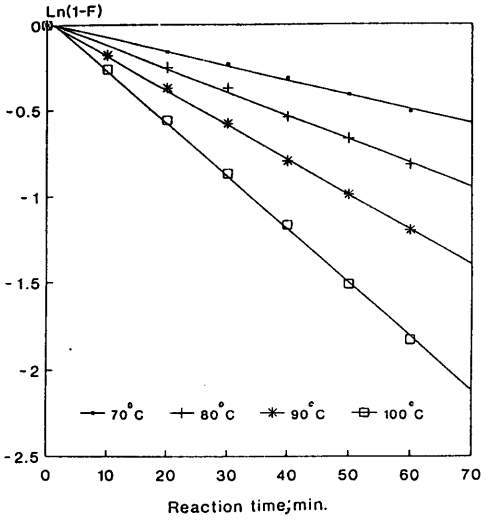


Fig (6): Variation of Ln(1-F) with time for 4-[^{131}I] iodophenyl alanine at different temperatures [1mg 4-iodophenyl alanine+1mg CuCl + 5µl Na^{131}I in 0.5 ml HCl (pH 3)]

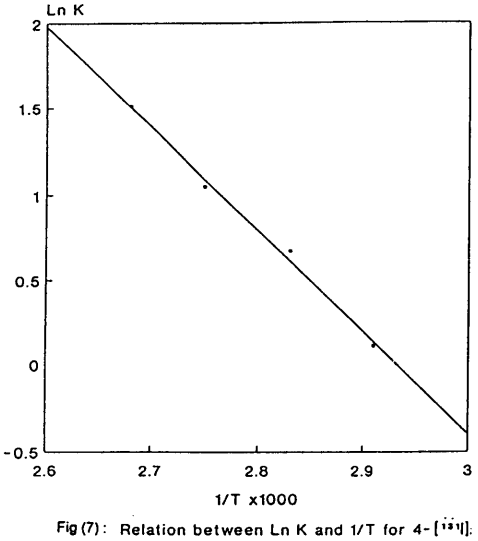


Fig (7): Relation between Ln K and 1/T for 4-[^{131}I] iodophenyl alanine

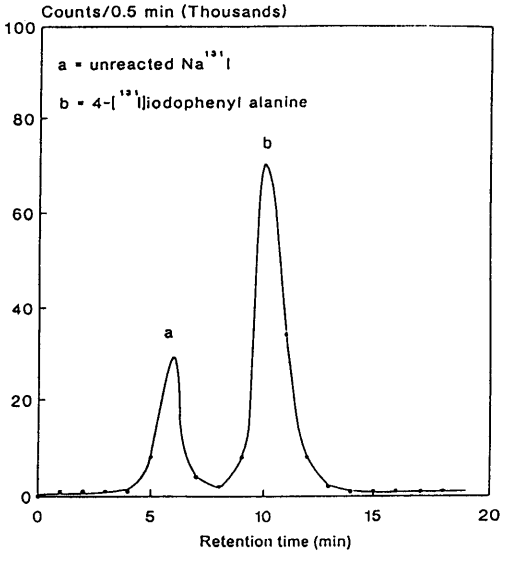


Fig (8): HPLC analysis of a reaction mixture of [1mg 4-iodophenyl alanine + 1mg CuCl + 10 mg sodium metabisulphite + 5µl Na^{131}I in 0.5 ml HCl (pH 3)]
Temp. = 100 °C Reaction time = 100 min.

REFERENCES

1) Lindly J. - Tetrahedron. 40 : 1433 (1984) .
2) Mertens J. , Vanryckeghem W. and Bossuyt A. - J. Labelled Compd. Radiopharm. 22 :89 (1985).
3) Wollny G. and Welzel W. - Isotopenpraxis 26 : 150 (1990).
4) Neves N., Paulo A. and Patricio L. - Appl. Radiat. Isot. 43 : 737 (1992) .
5) AL-Kolaly M.T., El-Bayoumy S., Raieh M. and El-Mohty A. - J.Radioanal. Nucl. Chem. 177 : 3 (1992)
6) Mertens J., Bossuyt- Piron C., DeGeeter F., Christianes L., Cautineau R., Guillaume M. and Leyssen J. - J. Nucl. Med. 30 : 926 (1989).
7) Mertens J., Gysemans M., Bossuyt-Piron C. and Thomas - J. Labelled Compd. Radiopharm. 28 : 731 (1991).
8) Battiste M. A., Strakowak L., Vanderbilt D. D., Visnik M., King R. W. and Nation S.L. - Tetrahedron Lett. 24 : 264 (1983).
9) Firnau G. , Garnet E.S., Chirakel R., Sood S., .Nahmias C. and Schrobilgen G. - Appl. Radiat. Isot. 37 : 669 (1986).
10) Coenen H.H., Bodsch W., Takahashi, K., Hossman K. A. and Stocklin G Nuclear Medizin 22 : 600 (1986).
11) Luurtsema G., Medema J., Elsinga P.H., Visser G.M. and Vaalburg W.- Appl.Radiat. Isot. 45 : 821 (1994).
12) Gieloue P. - Int. J. Appl. Radiat. Isot.. 35 : 1144 (1984).
13) Lambrecht R.M., Atkins H., Elias H., Fowler J.S., Lins S,S. and Wolf A.D.- J. Nucl. Med. 15 : 863 (1974) .
14) El-Wetery A.S., El-Mohty A.A., Ayyoub S., Raieh M. and Ghonaim A. - Arab Journal of Nucl. Sci. and Applications 30 : 117 (1997).
15) Namavari M., Satyamurthy N., Phelps M.E. and Barrio, J.R. - Appl. Radiat. Isot. 44 : 527 (1993).
16) Fortman D.I., Robbins P. J. and Sodo V.I. - Int. J. Appl. Radiat. Isot. 29 :449 (1978).
17) Moerlein S.M. - Radiochemica Acta 50 : 55 (1990).
18) Carey F.A. and Sundberg R. J. - Advanced Organic Chem., Plenum Press, New York (1977).
19) Mertens J., Vanryckeghem W., Bossuyt A. - J. Labelled Compd. Radiopharm. 21 : 303 (1984) .
20) Rossouw D. D. - Appl. Radiat. Isot. 43 : 1301 (1992) .
21) Dougan H., Lyster D. M. , Vincent J. S. and Rekela T. - J. Labelled Compd. Radiopham. 25 : 531 (1988).
22) Wheler O.H., Verler H.S., Teler L.A. and De jesus C.R. - J. Labelled Compd. Radiopharm. 7 : 40 (1971).
23) Kundo N. and Jaim S. K. - Physical Chemistry, Chand and Company, Ram Nagar, New Delhi (1987).

24) Liu B.B., Chang J., Sun J. S., Billing J., Steves A., Ackerhalt R., Molnar M. and Kung H.F. - J. Nucl. Med. 28 :360 (1987).
25) Liu B. B., Kung H.F. Billing J. and Blau M. - Nuclear Med. and Biology 14 : 69 (1987) .
26) Vedaneyev V. I., Gurvich L.V., Kondrayev V. A., Medvedev V. A. and Frankevich Y. L. - Bond energies, Ionization Potentials and Electron Affinities, Edward Arnold, London (1966).

