FLUORINE-18 LABELLING OF LOWER FATTY ACID ESTERS BY HETEROGENEOUS EXCHANGE ON GAS CHROMATOGRAPHIC COLUMNS

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SUMMARY

Fluorine-18 produced via the reaction chain $^{6}Li(n,\alpha)t$, $^{16}O(t,n)^{18}F$ by reactor irradiation of Li_2CO_3 was directly recovered in carrier-free form with a 90% yield by repeated extractions with water. A simple apparatus was used for simultaneous extraction and fast loading of an exchange resin with $^{18}F^-$. Dowex 1x4 in the acetate form was found to be best suited for a gas phase ^{18}F-for-Br exchange in α-bromofatty acid esters. The ^{18}F-loaded exchange resin was brought into a gas chromatographic column followed by an analytical column for the separation of the ^{18}F-labelled product from the unchanged bromine compound. The dynamic technique combines synthesis and purification and allows a fast and carrier-free production of ^{18}F-α-fluorofatty acid esters with boiling points up to about 300 °C. α-fluoroacetic acid ethyl ester and α-fluorovaleric acid methyl ester have been labelled carrier-free with yields of 64% (at 170 °C) and 39% (at 190 °C), respectively. Kinetic studies on the yield determining factors have also been carried out.

Key Words: Fatty Acid Esters, Radiopharmaceuticals, ^{18}F-Labelling, Exchange Reaction, Gas Chromatography, Fluorine-18

INTRODUCTION

The substitution of F-for-H in organic compounds can lead to analogues with almost unchanged or enhanced biochemical behaviour. This observation coupled with suitable decay characteristics of the positron emitter ^{18}F ($T_{1/2}$ = 109.87 min) stimulated efforts to synthesize ^{18}F-labelled biomolecules for in-vivo diagnosis [for a review cf. e.g. (1)]. Fluorine-18 also offers a convenient

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alternative as a tag in cases where synthesis with carbon-11 is difficult to achieve or where the half-life of ^{11}C ($T_{1/2}$ = 20.3 min) is too short for the medical study, provided the ^{18}F-label does not inhibit the involved biochemistry.

So far, no ^{18}F-labelled compound has been prepared in a carrier-free form, and the need for anhydrous and carrier-free fluorinating agents to be used in the synthesis of ^{18}F-radiopharmaceuticals is obvious. We have applied the heterogeneous exchange on gas chromatographic columns to achieve this goal in the case of some simple fatty acids. The method of labelling volatile compounds by this technique was first reported in 1960 by STÖCKLIN and coworkers (2-4). New data dealing with the exchange of ^{82}Br and ^{18}F/alkyl-halides and tritium/hydrocarbons were added by STÖCKLIN (5) in 1964. A complete summary of this type of exchange can be found in a review by ELIAS (6). An interesting approach has recently been used by ROBINSON (7) for the synthesis of ^{18}F-labelled α-fluoro-fatty acid esters. The author sealed and heated an $(^{18}F^-)F^-$-loaded exchange resin along with the substrate in a closed ampoule. We have used exchange resins to achieve an ^{18}F-for-Br exchange in α-bromofatty acid esters in the gas phase. The virtue of this technique is that it can produce carrier-free labelled products in a mild and fast way, combining exchange and purification steps.

EXPERIMENTAL

Preparation of Fluorine-18 and the Labelled Resin

20-40 mg enriched Li_2CO_3 (95% lithium-6) carefully dried were vacuum-sealed in quartz ampoules and then irradiated at a thermal neutron flux density of $6.7 \cdot 10^{13}$ $cm^{-2}sec^{-1}$ for 3 to 4 hours. The irradiated ampoule was broken into very small pieces while immersed in 5 ml of water. A simple apparatus (Fig. 1) for a combined extraction of ^{18}F and labelling of the resin in one cycle has been

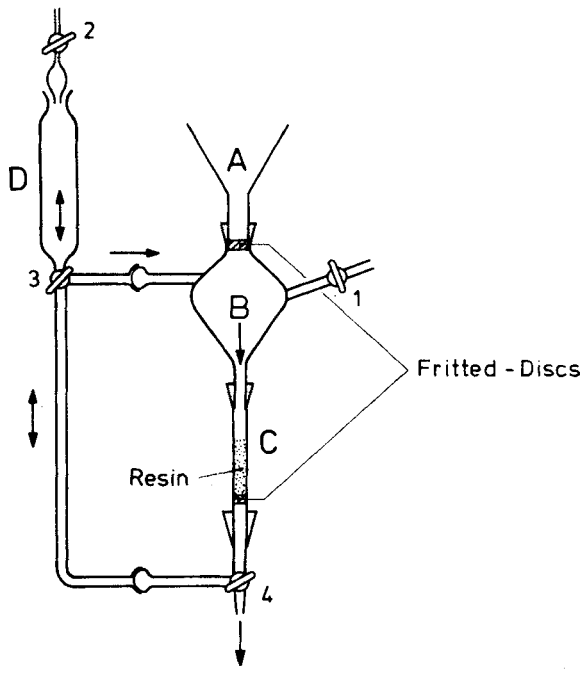

Fig.1. Apparatus for water extraction of ^{18}F from Li_2CO_3 and for loading the resin with ^{18}F-ions.

designed and used in this study. The target along with the broken ampoule was brought into funnel A together with another 5 ml of water. For studying the extraction behaviour of carrier-free ^{18}F directly with water a number of collecting flasks were attached below the bulb B and the active solutions collected in each of these flasks by applying a slight vacuum at point 1. Care was also taken that Li_2CO_3 did not itself pass through the fritted disc.

Breaking the ampoule and the extraction of 10 ml of water solution was completed within five minutes. Each of the subsequent extractions was performed with 5 ml of water and consumed one minute. The extraction procedure was repeated six times after which dil. HCl was added and all of the Li_2CO_3 dissolved in it. This acidic solution along with other washings of the bulb B with water

containing fluoride-carrier was collected in another flask. The activities of the individual fractions and of Li_2CO_3 solution were then measured. Curve A in Fig. 2 gives the % recovery of carrier-free ^{18}F in each extraction while curve B shows the cumulative yield. Nearly 80% of ^{18}F activity can be separated in 5 minutes in the first extraction, and 98% of the activity can be collected in 35 ml of water within 10 minutes. In places where no cyclotron is available, this arrangement allows a simple and fast extraction procedure for processing carrier-free ^{18}F from reactor irradiated Li_2CO_3. Irradiation of Li_2CO_3-slurries (8) in the core of a reactor requires much more precautions as compared to irradiation of dried Li_2CO_3 and does not provide any advantages. The tritium content in the final ^{18}F solution does not interfere with the preparation of the labelled resin and the exchange in the present work. If required it can be eliminated by repeated evaporation of the processed solution.

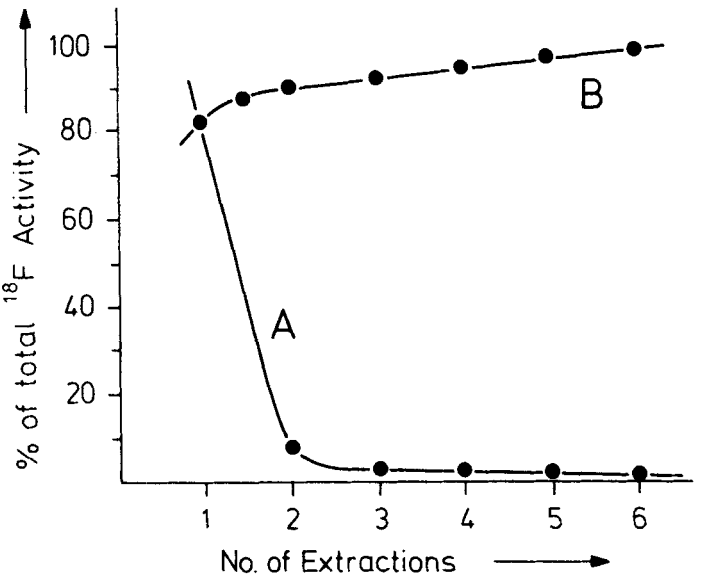

Fig.2. ^{18}F activity recovered vs. no. of extractions.

The process of labelling the anion loaded Dowex 1x4 (200-400 mesh) filled in column C was carried out after breaking the irradiated quartz ampoule and bringing the Li_2CO_3 into funnel A together with 10 ml of H_2O. Vacuum was applied at point 2, and the ^{18}F solution after passing through the resin was collected in column D. After repeated extractions the funnel was taken away and all the solution from column D was immediately transferred to the bulb B by opening the stopcock 3 for recirculation of ^{18}F solution through the resin. The labelled resin was washed three times each with water, methanol and diethylether; in each run 5 ml of wash liquid was used.

The hydroxide, chloride, bromide, iodide, acetate and oxalate form of the resin (Dowex 1x4, 200-400 mesh) were prepared and tested in the columns for studying the exchange character of these species with α-bromine of fatty acid esters. Inorganic supports were also tried because of their stability at higher temperatures. For preparing $^{18}F^-$-alumina the pH of ^{18}F solution was adjusted to bromothymol blue indicator before passing it through alumina already packed in column C (Fig. 1). Fire brick (0.4-0.5 mm) was mixed with ^{18}F solution and the slurry thus obtained was evaporated to dryness. Anhydrous zirconium oxide (Biorad Ion-exchange Crystals H20-1, 20-50 mesh) was also filled in column C and ^{18}F solution recycled through it.

The ^{18}F-loaded resin after drying for five minutes with hot air was packed into an 80 cm gas chromatographic steel column (6 mm i.d.) and served as the stationary phase. The flow of the carrier gas was continued and the temperature of the column raised gradually. When the system was stabilized micro-amounts of an α-bromofatty acid ester were injected. Different experimental conditions such as changes in temperature and the contact time of the substrate with the active resin were studied. In order to obtain prolonged and controlled contact times for the exchange, the flow of the

carrier gas was stopped by closing inlet and outlet of the exchange column by means of a 4-way valve. The adsorption complex formed and decomposed on the resin thus leading to the labelled product which was then analysed as it passed through the separation column attached in-line just after the exchange column. The activity assay of the labelled esters leaving the separation column was carried out by a radio gas chromatographic technique (9). For the separation of the α-fluorofatty acid esters from the unconverted α-bromofatty acid ester a 240 cm long (6 mm i.d.) column filled with 20% SF-96 on Chromosorb W (60-80 mesh) was used at different temperatures. This analytical separation column immediately followed the exchange column which was kept at a different thermostat at a different temperature. The experimental set-up has been described in detail in previous papers (4,5,10).

RESULTS AND DISCUSSION

The ideal characteristics required from the anionic species on the resin for carrier-free synthesis was that it should efficiently be replaced by carrier-free $^{18}F^-$ but should itself not exchange with α-bromine of the ester. This was desirable, since in the case of carrier-free $^{18}F^-$ only a negligibly small amount of the total anions of the exchange resin can be replaced. Thus, during the passage of the α-bromofatty acid ester (RBr) the major exchange occurs with the inactive counter ion A^-

$$A^- + RBr \rightleftharpoons RA + Br^-, \qquad (1)$$

giving rise to a substituted product RA, in addition to the desired exchange

$$^{18}F^- + RBr \longrightarrow R^{18}F + Br^- \qquad (2)$$

F-For-Br Exchange in α-Bromofatty Acid Esters (Fluoride Resin)

The F-for-Br exchange in α-bromofatty acid esters on a column loaded with ^{18}F-labelled fluorine ions gives rise to the non-carrier-free α-fluorofatty acid ester. Fig. 3B shows the chromatogram of the products detected by the thermal conductivity cell after F-for-Br exchange in α-bromovaleric acid at 180 °C. Since the resin was loaded with macroscopic amounts of F^--ions the major mass peak is due to inactive ester which appeared with the same retention time as the authentic α-fluorovaleric methyl ester, taking into account the inherent delay time. The $^{18}F^-$ activity of the products was simultaneously measured and Fig. 3A shows the response of NaI(Tl) detector. From the area under the mass peaks it can be estimated that for a contact time of only 1 minute more than 40% of α-bromovaleric ester was converted to the ^{18}F-labelled α-fluoro product.

In general F-for-Br exchange should also occur when higher fatty acid esters are injected. An extreme case of palmitic acid ethyl ester (B.P. 390 °C) was also tried. Microvolumes of $CH_3(CH_2)_{14}CHBrCOOC_2H_5$ were injected into a column of $^{18}F^-$ fluoride resin. With a contact time of 10 minutes at 190 °C the exchange was obtained but the yield was very low. To achieve better yields both the temperature and contact time are to be increased. While the former leads to the decomposition of the resin the latter is governed by the half-life of ^{18}F. However, the method should be easily applicable to the labelling of fatty acid esters with boiling points up to about 280 to 300 °C.

Synthesis of Carrier-free α-^{18}F-Fluorofatty Acid Esters

Out of all the resins and inorganic materials tested the acetate form of Dowex 1x4 was finally selected (10) for carrier-free preparation of the α-fluorofatty acid esters because the α-bromine

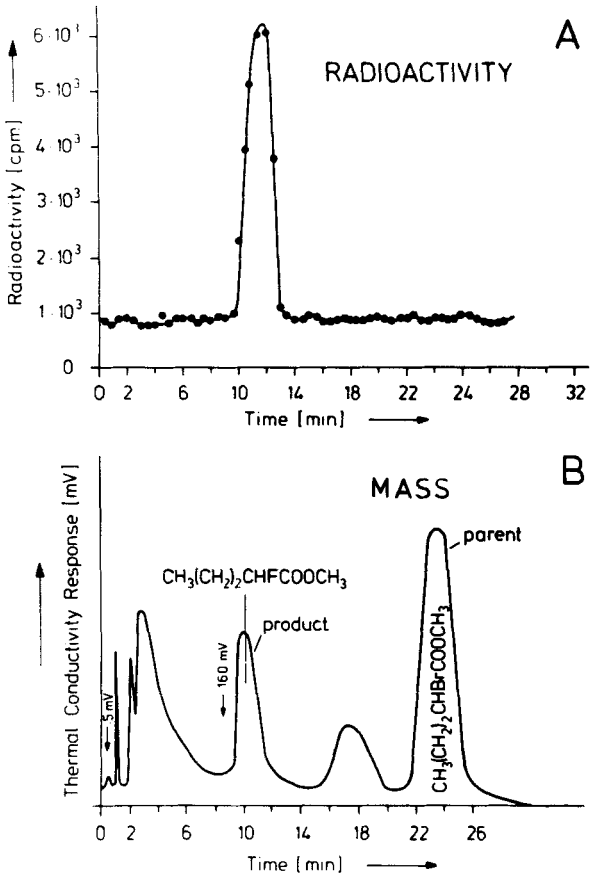

Fig.3. Radio gas chromatogram after F-for-Br exchange in α-bromovaleric acid.

Exchange column : 80 cm Dowex 1x4 (100-200 mesh), ^{18}F-labelled fluoride form

Temperature : 180 °C

Contact time : 1 min

Analyt. column : 240 cm SF-96 (20%) on Chromosorb W (60-80 mesh)

Flow rate : 100 ml He/min

10 µl $CH_3(CH_2)_2CHBrCOOCH_3$ injected

A. ^{18}F-radioactivity recording of 1 min-fractions

B. Thermal conductivity recording of macroscopic products

exchanged most effectively with carrier-free $^{18}F^-$-ions. However, the inactive acetate ions of the resin are also exchanged and the corresponding inactive acetato product is formed. Processing of the ^{18}F activity, labelling of the acetate resin with it (1-2 ml wet volume of the resin), filling the exchange column and drying the resin at elevated temperature could all be completed within one half-life (100 min), and injections of α-bromofatty acid ethyl ester then started. In the absence of a mass peak in these carrier-free preparations the formation of the ester was indirectly confirmed by the retention time of the activity peak obtained from the radio gas chromatographic data.

(a) Effect of temperature on ^{18}F-for-Br exchange

The effect of temperature on the % exchange yield of carrier-free $CH_2{}^{18}FCOOC_2H_5$ was studied at 140 and 170 °C. The % exchange is related to "exhaustion of the column", i.e. depletion from ^{18}F by the equation (4,5)

$$A_n = A_1 \cdot k^n \qquad (3)$$

$$(1-k)\cdot 100 = \%\ \text{exchange} \qquad (4)$$

where A_1 is the ^{18}F-activity of carrier-free ester after the first injection and A_n its activity after the n_{th} injection.

In Fig. 4A the exhaustion curves are shown for 170 °C and for 140 °C. The exchange yield of carrier-free $^{18}FCH_2COOC_2H_5$ increased from 49% at 140 °C to 64% at 170 °C. The corresponding exhaustion curves for the exchange in α-bromovaleric acid methyl ester at 160 °C and 190 °C are shown in Fig. 4B.

(b) Effect of contact time on ^{18}F-for-Br exchange

Five columns were packed with batches of ^{18}F-labelled resin from the same stock. Each column was used for studying the effect of contact time on the yield of carrier-free $CH_2{}^{18}FCOOC_2H_5$. Fig. 5A shows a plot of the relative yield versus the contact time. As expected from the first order exchange law for this type of

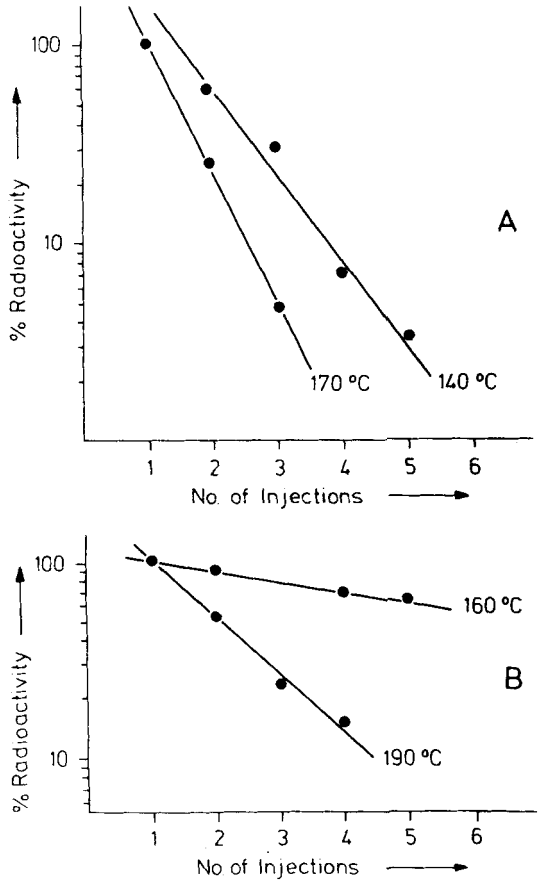

Fig.4. Temperature dependence of column exhaustion in ^{18}F-for-Br exchange (cf. eq. 3 and 4)

Exchange column : 8 cm Dowex 1x4 (100-200 mesh) acetate form loaded with carrier-free $^{18}F^-$

Analyt. column : 240 cm SF-96 on Chromosorb W (60-80 mesh)

He-flow rate : 100 ml/min

5 µl of α-bromofatty acid ester used in each injection

A. $^{18}FCH_2COOC_2H_5$ from ^{18}F-for-Br exchange in $BrCH_2COOC_2H_5$ at 140 and 170 °C

B. $CH_3(CH_2)_2CH^{18}FCOOCH_3$ from ^{18}F-for-Br exchange in $CH_3(CH_2)_2CHBrCOOCH_3$ at 160 and 190 °C

Relative yields given in % ^{18}F-radioactivity (activity of first injection = 100)

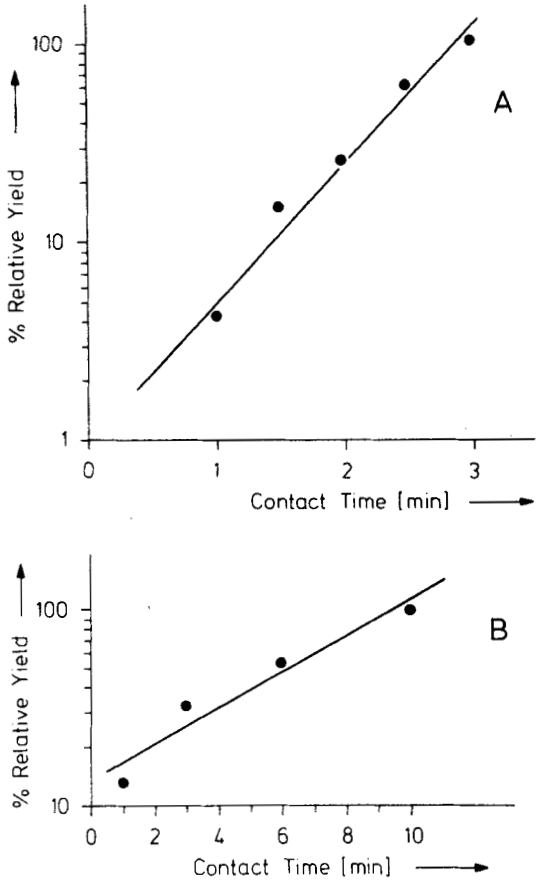

Fig.5. Effect of contact time on ^{18}F-for-Br exchange.

Exchange column : 8 cm (A) and 16 cm (B) Dowex 1x4 (100-200 mesh), acetate form, loaded with carrier-free $^{18}F^-$

Temperature : 160 °C (A) and 190 °C (B)

Analyt. column : 240 cm SF-96 on Chromosorb W (60-80 mesh)

10 µl (A) and 20 µl (B) of α-bromofatty acid ester injected each time

Relative yields in % of ^{18}F-activity (activity at longest contact time = 100)

A. ^{18}F-for-Br exchange in $BrCH_2COOC_2H_5$

B. ^{18}F-for-Br exchange in $CH_3(CH_2)_2CHBrCOOCH_3$

exchange (4,5), the yield plotted in a semi-logarithmic scale versus the contact time gives a straight line. The corresponding dependence of the exchange yield for the case of α-bromovaleric acid methyl ester is shown in Fig. 5B.

(c) Dependence of ^{18}F-for-Br exchange on the amount of substrate injected

The study was conducted under similar conditions as those for observing the effect of contact time except that the amount of substrate in each injection was changed. The contact time was not prolonged by closing the exchange columns but was determined by the natural residence time given by the flow rate (100 ml/min), thus it was considerably shorter and the % exchange is much smaller. The results are shown in Fig. 6 for the bromoacetic acid ethyl ester (A) and the α-bromovaleric acid ester (B) at 170 and 190 °C, respectively. With increasing amount of injected esters the exchange yield first increases then reaches a saturation, as expected on the basis of classical adsorption phenomena.

Mechanistic Aspects and Conclusion

If the heterogeneous exchange proceeds via a classical nucleophilic substitution a 100% inversion should be expected. In order to test this, we have also studied in our laboratory (11) the F-for-Cl exchange in the diastereomers meso- and rac-2,3-dichlorobutane at 100 °C on Dowex 1x4 (200-400 mesh). It was observed that the Cl-for-F exchange in meso-2,3-dichlorobutane leads exclusively to threo-2-chloro-3-fluorobutane and that of rac-2,3-dichlorobutane to erythro-2-chloro-3-fluorobutane. The results indicate that at least the Cl-for-F exchange in these alkyl halides proceeds with complete inversion under the above mentioned conditions. It is, however, interesting to note that the ^{82}Br-for-Br exchange in rac-2,3-dibromobutane occured with almost complete retention (98%) when the $^{82}Br^-$-ions were adsorbed on fire brick (0.2-0.3 mm) at

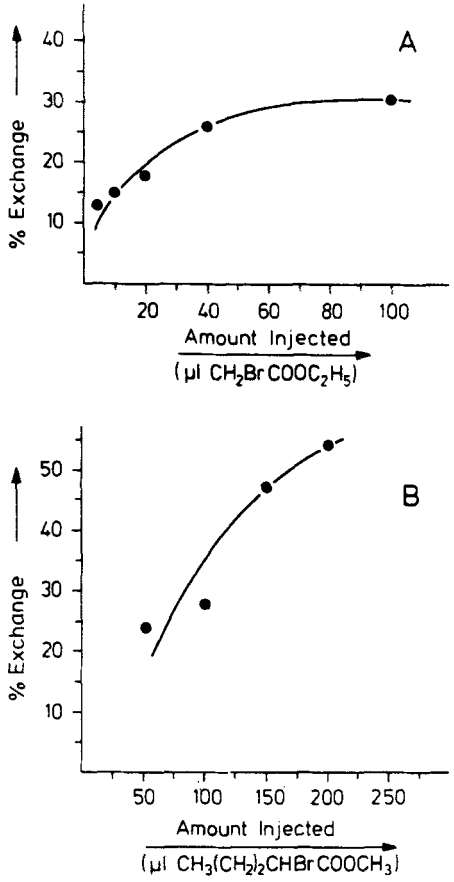

Fig.6. Dependence of the ^{18}F-for-Br exchange on the amount of substrate injected.

Exchange column : 8 cm (A) and 16 cm (B) Dowex 1x4 (100-200 mesh), acetate form, loaded with carrier-free $^{18}F^-$

Temperature : 170 °C (A) and 190 °C (B)

He-flow rate : 100 ml/min

Analyt.column : 240 cm SF-96 on Chromosorb W (60-80 mesh)

125 °C (12). Obviously, the stereochemistry of this heterogeneous halogen exchange depends strongly on the environment of the halide ions on the solid support. Besides carrier-free labelling with short-lived halogen isotopes the technique might also be of interest for stereoselective syntheses and possibly as a probe for surface characterization.

The gas phase chromatography method is, of course, limited to volatile compounds and higher fatty acids cannot be easily prepared. Exchange columns suitable for higher temperatures, however, may allow an extension of the method. In any case, ^{18}F-labelled fluoro-fatty acids can be converted to other more complex derivatives through a multiplicity of organic reactions as was suggested by Robinson (7), e.g. ^{18}F-fluoroacetyl chloride has considerable potential for use in the rapid preparation of a wide variety of compounds, such as fluoro-acetyl salicylic acid (an aspirin analogue) and cholesteryl fluoroacetat

It was also suggested (7) that the lipid-soluble compounds such as α-fluorocarboxylates and 2-fluoroethanol may prove useful for the evaluation of regional brain perfusion due to the apparent absence of a blood-brain barrier for many of such substances (13,14). The medium and long chain α-halofatty acids are expected to be potential agents for myocardial imaging (7,15) and for in-vivo studies of heart muscle metabolism (16). Due to their relatively non-specific, high tissue extractability, fluoroacetate and 2-fluoroethanol are promising agents for the measurement of fractional cardiac output (7).

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