FAST LOW TEMPERATURE ULTRASONIC SYNTHESIS AND INJECTION READY PREPARATION OF CARRIER FREE 17-I-123-HEPTADECANOIC ACID

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SUMMARY

17-I-123-heptadecanoic acid is prepared carrier-free from 17-Br-heptadecanoic acid in methyl ethyl ketone at 100°C. A quantitative labeling yield is obtained within 20 minutes using a 50 KHz ultrasonic bath. Sonication allows the presence of water up to 7.5 % v/v. The pattern shown by the plot of the kinetic curves is typical of two consecutive reactions: the first being the dissociation of a radioactive side product, the second a I^- for Br nucleophilic exchange. The pure carrier-free radioiodinated fatty acid is obtained by HPLC separation and is used clinically in a sterile 6% human serum albumine solution. The quality control of the injection ready solution is based on the use of a RP18 Sep-Pak^R cartridge.

Key-words: 17-I-123-heptadecanoic acid, high pressure liquid chromatography, carrier-free, kinetics, injection ready radiopharmaceutical.

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INTRODUCTION

ω-I-123 labeled fatty acids containing an odd number of carbons are proved to be valuable agents for imaging the myocardium and the liver (1,2,3,4,5).

The use of the short-lived but expensive I-123 for the non-isotopic exchange labeling of 17-Br-heptadecanoic acid requires both a high reaction rate and a high reaction yield.

Apart from the boron chemistry described by Kabalka et al. (6,7), the methods developed for the ω-labeling of fatty acids can be divided into two main trends. One prefers low reaction temperatures, such as refluxing conditions. These are time consuming and the yields do not exceed 80% (8,9,10). The other one opts for high temperatures or hot melts in order to increase the reaction rate. The latter suffers from the thermal degradation of the products involved in the labeling reaction, resulting in a critical reaction time and/or decreased yield (11,12,13,14). The aim of this paper is to present a gentle, but nevertheless fast synthesis of 17-I-123-heptadecanoic acid (*IHA), based on the use of a commercially available 50 KHz ultrasonic bath at a reaction temperature of 100°C.

EXPERIMENTAL

Materials

The iodide-123 is supplied by IRE (Fleurus, Belgium) in an aqueous solution (0.5 mCi/µl H_2O). The 17-Br-heptadecanoic acid is obtained from BIPHARCO (Belgium). The products and solvents used for the synthesis are all p.a. grade (Merck). The methanol used in the chromatographic procedure is HPLC grade (Merck) and the water is of double distilled quality.

Apparatus

The ultrasonic bath is a 50 KHz Bransonic 321 provided with an external heating system (Sinus - Zeiss Holland, 1000 Watt). The reaction vials are 5 ml mini vials with conical cavity supplied by Chrompack, but the original screw-cap is replaced by a custom-made metallic one and a rubber septum.

The HPLC system consists of a Waters U6K injector (2 ml loop), a Waters M 6000 A pump, a Waters refractive index (RI) detector coupled to a γ-scintillation detector (a coiled PTFE capillary mounted above the surface of a flat 3" NaI (Tl) detector crystal (Ortec) connected to Ortec electronics (high voltage, supply, single channel analyser, amplifier, ratemeter)) and a HP 3380 A integrator.

Mass-spectrometry coupled gas chromatography analysis (GC-MS): Finnegan 3200 GC-MS coupled to a INCOS data system. Column: Chrompack Sil.5, 3% on Chromosorb WHP80/100. Temperature programmation: 50-200°C at 4°C/min. heating rate.

Ultrasonic synthesis of 17-I-123-heptadecanoic acid

5 mg 17-Br-heptadecanoic acid (1.1410^{-5} mol) (BrHA), 2 ml of methyl ethyl ketone (MEK), 2 µl of a freshly prepared saturated thiosulfate solution and the I-123 solution (1 - 60 mCi) are successively added to and mixed in the mini vial. The vial is placed in the ultrasonic bath containing water kept at its boiling point. After 20 minutes the reaction mixture is evaporated to dryness by flushing with N_2 through a double needle system. The MEK vapour is led through a 1 M thiosulfate solution to control the accidental formation of radioactive I_2.

Chromatography

After evaporation 2 ml of MeOH is injected into the reaction vial to solubilize the reaction mixture. The entire volume is injected into the HPLC system as fast as possible.
HPLC separation: 16 x 250 mm Chrompack Lichrosorb 10 µ -RP18 column
Eluent: MeOH/H_2O/Acetic Acid (93/6.5/0.5), flow rate: 8 ml/min.

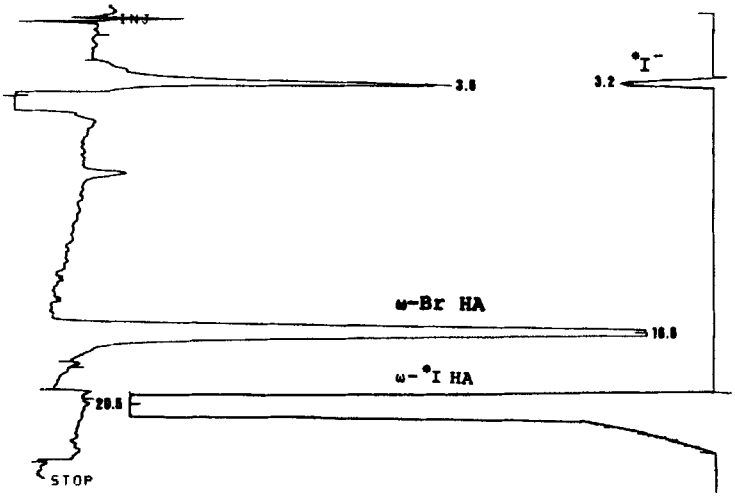

Fig.1. Left trace: chromatogram obtained with RI detector showing as the major peak the BrHA (retention time of 16.6 min.). Right trace: radiochromatogram representing the *IHA (retention time of 20.5 minutes).

The carrier-free 17-I-123-heptadecanoic acid is collected in a vial after complete chromatographic separation from the other products (fig.1).

Injection ready preparation

The HPLC eluent is evaporated almost to dryness by passing a stream of preheated (100°C) nitrogen. Then 300 µl of ethanol (Merck p.a.) are injected while rinsing the walls. 1.7 ml of 6%

human serum albumine (HSA) in physiological saline are rapidly injected with vigourous vortexing. This solution is sterilized by means of a 0.22 µ filter (Millipore, Millex GV) which has been pretreated by passing a saturated Br HA/6% HSA solution and rinsed with physiological saline. Experiments with 17-Br-82- heptadecanoic acid and 17-I-123-heptadecanoic acid have shown that the fatty acids adsorbed on the filter during presaturation, do not interfere with the required radiopharmaceutical.

Presaturation allows a filtration yield of at least 95% to be obtained. The overall preparation yield of the radiopharmaceutical, i.e. synthesis, HPLC separation and sterilization, amounts to at least 85% and requires 90 minutes.

Quality control of the injection ready radiopharmaceutical

Quality control is carried out by two methods. One method is based on the use of a Sep-Pak^R C-18 reversed phase cartridge (Waters Associates). 1 ml of HPLC eluent is vigourously mixed for 30 seconds with 50 µl of an injection ready solution of the fatty acid. The resultant suspension is passed through the Sep-Pak^R cartridge which is then rinsed with 1 ml of H_2O. *IHA released from the serum proteins by denaturation is retained on the packing, while free I^--123 passes through completely. In successful preparations, more than 97% of the activity remains on the filter. The other method is a modified protein bound iodide (PBI) method based on the use of Iobeads Resin (Technicon T 15-0150-28)(15) where the activity of *IHA in the supernatant 6% HSA solution is measured after sedimentation of the beads. As a criterion for a good quality preparation we accept that more than 95% of the activity should remain in solution.

RESULTS AND DISCUSSION

Advantages of ultrasonification

Figure 2 plots the reaction yield η in % (activity of *IHA/ total activity of I-123 X 100) as a function of time when equal reaction mixtures in the same type of reaction vial (see experimental) are submitted to the ultrasonic bath method and to a conventional hot water bath, both using boiling water.

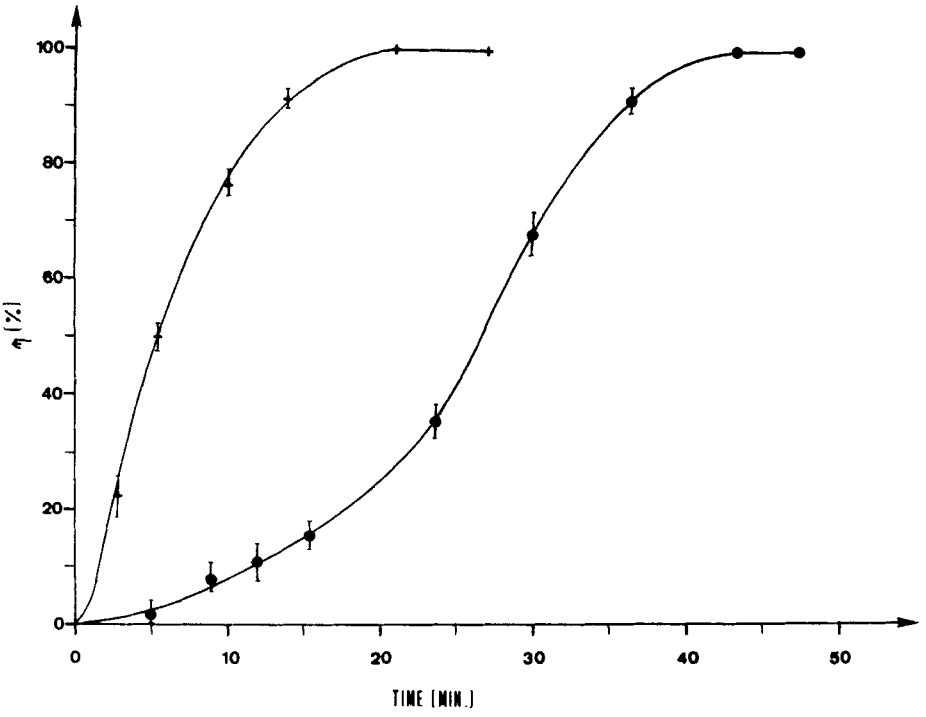

Fig.2. Yield η (in %) of *IHA as a function of time (mean of three experiments). + : ultrasonic bath; ● : water bath. Reaction conditions: 5 mg BrHA, 2 ml MEK, 2 µl saturated thiosulfate solution, 152 µl H_2O, 0.5 mCi I^--123. Reaction temperature: 100°C.

As shown the ultrasonic system allows a quantitative reaction yield to be obtained within 20 minutes, even in the presence of 150 µl of water, while the reaction occuring in the hot water bath is characterized by a much slower rate. The fact that in our hands, the latter method gives a quantitative yield after a reasonable reaction time (50 min.), contrary to the methods using refluxing conditions (7,8,9,10), can be explained by the higher reaction temperature (100°C) reached in the pressurized vial.

The principal advantage of the ultrasonic method is assumed to be a rapid and high degree of emulsification and/or solubilization of products and solvents involved in the reaction, thus increasing the collision frequency factor and the reaction rate. The presence of water is allowed in contrast to earlier described methods where freshly distilled and dried solvents are to be used (9) or water has to be evacuated by lyophilisation before starting the reaction (13).

Radioactive side product

For the ω-iodination of fatty acids by halogen exchange reactions, ketones are prefered to other solvents (9,17).In our hands, when adding radioiodide (I-123 or I-131) to MEK, a radioactive side product is quantitatively formed within 1 minute at room temperature. After submitting the MEK solution, containing the side product, to the ultrasonic treatment at 100°C, HPLC analysis showed the quantitative recovery of the radioactive side product after evaporation of the MEK. Laufer et al. (13) also report the formation of radioactive side products lowering the labeling yield when using MEK or acetone as solvent for the ω-labeling of fatty acids by $^{*}I^{-}$ for Br exchange. According to these authors, the use

of freshly distilled MEK did not avoid the formation of the side product. The use of ultra pure GC grade MEK (Merck) was tried, but resulted also in the formation of the radioactive side product.

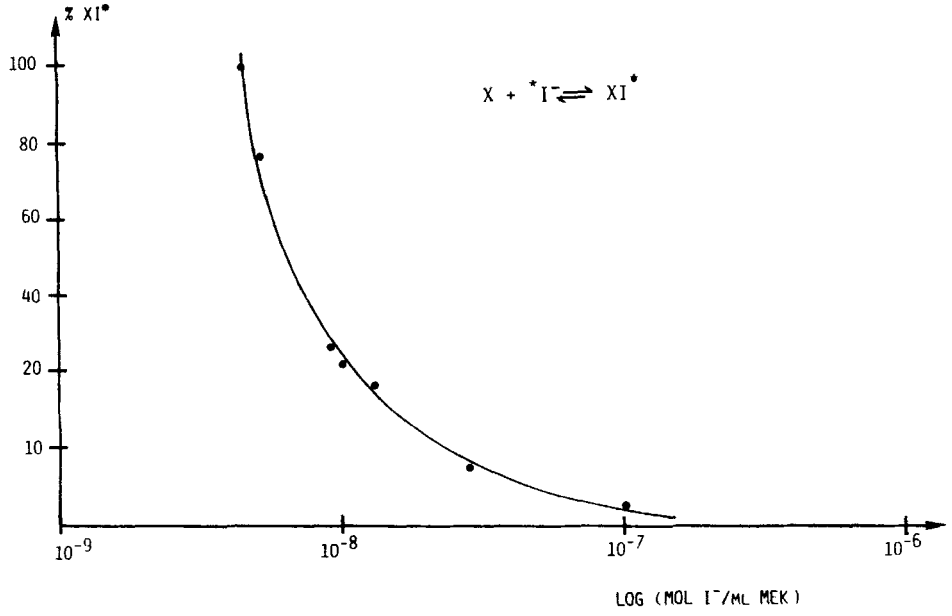

Fig.3. Decrease of radioactive side product X*I as a function of cold I^- added 10 minutes prior to the radioiodide. Reaction conditions: x ml MEK + y ml of 10^{-7} mol KI in MEK solution (x + y = 2 ml), after 10 minutes, 0.1 mCi of I^--123 are added. Reaction temperature: 30°C, reaction time: 5 minutes.

We have shown that by the addition of cold iodide to the MEK, 10 minutes prior to the radioiodide, the amount of radioactive side product decreases in the reaction mixture (fig.3). Assuming a reaction of the type $X + {}^{*}I^- \rightleftharpoons X^{*}I$, the concentration of the impurity leading to the formation of the iodinated side product is calculated to be 10^{-8} mol/ml MEK. GC-MS analysis showed the side product to be for the larger part butyliodide.

The addition of 10^{-7} mol of cold iodide prevents the formation of the side product, but results in a non-carrier free preparation. It was experimentally established that the addition of thiosulfate, which had as a prime role to prevent the formation of I_2, allows to obtain a labeling yield higher than 99%. No radioactive side product was observed anymore at the end of the reaction.

Kinetics of reaction

As published in an earlier paper (16), maximum yields are obtained from 2.5 mg of BrHA per ml of methyl ethyl ketone.

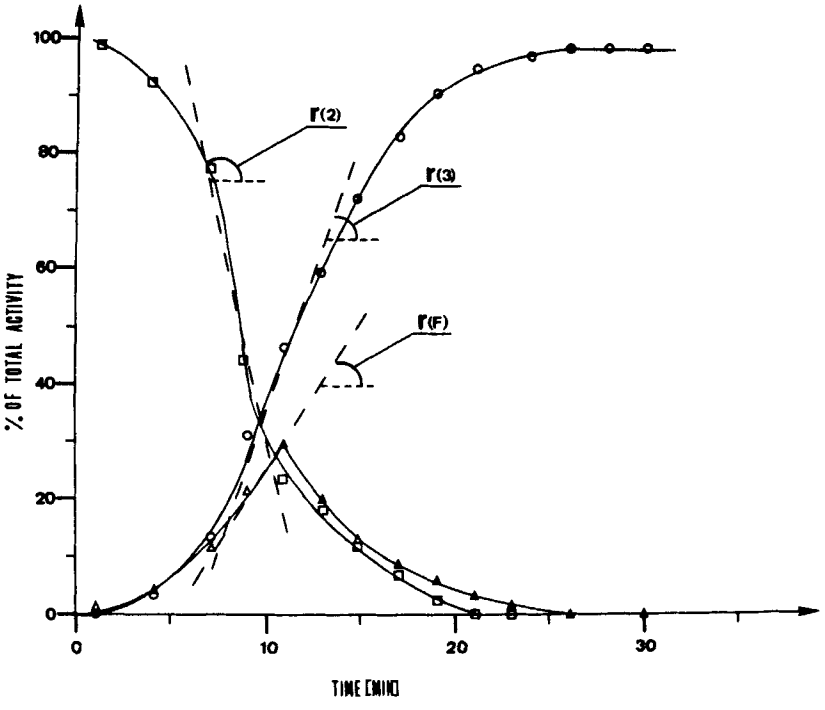

Fig.4. % of total activity of radioactive entities as a function of time (mean of 3 experiments).
□ : X*I ; △ : *I⁻ ; ○ : *IHA.

Plotting the radioactive entities as % of the total activity as a function of time (fig. 4) shows the radioactive side product X*I to be formed quantitatively within the first minute, i.e. once the radioiodide is added to the reaction mixture and in the beginning of the ultrasonic treatment. From that moment the reaction is characterized by the decrease of that side product resulting in the formation of both *IHA and free radioiodide $*I^-$. From $t_{50\%}$ of the *IHA formation, $|*I^-|$ decreases and the iodide is quantitatively consumed to the advantage of the iodination of the fatty acid.

The evolution of the concentration of the radioactive products as a function of time shows a pattern which is typical for two consecutive reactions (18, 19). This proves that the evolution of the reaction is more complicated than a single step $*I^-$ for Br nucleophilic substitution reaction as assumed until now in literature (9,11,20). The results shown in figure 4 allow the overall labeling reaction to be represented as:

$$1^\circ \quad X + *I^- \rightleftharpoons X*I \qquad (1)$$

Very fast formation of the radioactive side product once the radioiodide is added to the reaction mixture at room temperature.

$$2^\circ \quad X*I \rightleftharpoons X + *I^- \qquad (2)$$

Dissociation due to the reaction conditions during the ultrasonic treatment at 100°C (shift to the left of the equilibrium mentioned in reaction (1)).

The reaction rate $r_{(2)} = -\frac{d|X^*I|}{dt} = \frac{d|^*I^-|_{(2)}}{dt}$ (2')

3° $^*I^- + BrHA \rightleftharpoons {}^*IHA$ (3)

Formation of the 17-I-123 heptadecanoic acid by $^*I^-$ for Br nucleophilic exchange on the substrate.

The reaction rate $r_{(3)} = \frac{d|^*IHA|}{dt} = -\frac{d|^*I^-|_{(3)}}{dt}$ (3')

Because of conservation of the I-123 activity the mass balance of the radioiodide per unit of time can be written as :

$$-\frac{d|X^*I|}{dt} = \frac{d|^*IHA|}{dt} + \frac{d|^*I^-|_f}{dt} \quad (4) \text{ with}$$

$\frac{d|^*I^-|_f}{dt} = \frac{d|^*I^-|_{(2)}}{dt} - \frac{d|^*I^-|_{(3)}}{dt} = r_f$ being (5) the concentration

change of free $^*I^-$ per unit of time.

As the % of the total activity is proportional to the absolute concentration of each of the radioactive entities shown in figure 4, the slope of the curves in the region of interest for kinetic interpretation (between 20 and 80%) is proportional to the involved reaction rates. The experimental values obtained from figure 4 for $r_{(2)}$, $r_{(3)}$, $\frac{d|^*I^-|_f}{dt}$ are respectively 14.7, 9.3 and

5.3 %/min. Substitution of these values in equation (4) shows the mass balance to be accurate within the experimental errors. Taking into account the values of the reaction rates $r_{(2)}$ and $r_{(3)}$, it can be concluded that $*I^-$ for Br nucleophilic exchange (reaction (3)) is the rate determining step.

Quality of the product - Clinical applications

Table 1 records the results of the quality control experiments on the injection ready *IHA solution stored at 4°C. The product is stable for at least four days.

The *IHA, produced as described above, is used routinely in clinic for heart and liver studies. 1-2 mCi of *IHA are injected intraveneously. The carrier-free *IHA, besides being a valuable radiopharmaceutical for the study of the myocardial metabolism, is also found to be a good hepatocytic scanning agent (21).

Table 1. Results of quality control.

DELAY(days)	0	1	2	3	4	5	6	7
ACTIVITY IHA / TOT. ACTIVITY	0.97	0.97	0.97	0.96	0.96	-	-	0.82

CONCLUSION

The described reaction conditions, using a commercially available ultrasonic bath, allow to obtain labeling yields of the heptadecanoic acid with I-123 of more than 99%. Sonication permits

the presence of considerable amounts of water. The 17-I-123-heptadecanoic acid is recovered carrier free after HPLC separation.The overall yield of the radiopharmaceutical is at least 85%. MEK, which is an appropriate solvent for nucleophilic halogen exchange reactions, yields inevitably a radioactive side product when adding radioiodide to the reaction mixture. It was experimentally established that in presence of thiosulfate, the labeling reaction proceeds quantitatively. The reaction is shown to be more complicated than currently assumed and passes through the stage of a radioiodinated side product, although the I for Br exchange remains the rate determining step. The final product was proven to be stable for at least four days and to be clinically reliable.

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