A SIMPLE METHOD FOR THE PREPARATION OF CARRIER FREE I-LABELLED 4-IODOANTIPYRINE

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ABSTRACT

A simple new method for synthesis of a carrier free (^{131}I)-4-iodoantipyrine has been developed. The procedure uses dry column chromatography on silica gel, where reaction between iodine-131 and antipyrine is carried out simultaneously with elution of (^{131}I)-4-iodoantipyrine. The labelling yield is around 80 per cent; radiochemical purity of the final product exceeds 98 per cent.

Keywords: Labelled 4-Iodoantipyrine, Carrier Free Labelling, Dry Column Labelling.

INTRODUCTION

4-iodo-antipyrine (4-IAP) or 4-iodo-2,3-di-methyl-1-phenyl-3-pyrazol-5-one (2) has been used for autoradiographic measurement of the regional cerebral blood flow in animals (1). The 4-IAP used in the measurements was labelled with a radioactive iodine (2,3) or carbon-14. An iodine-labelled material is of particular interest in double-isotope labelled experiments, where regional glucose utilization and regional blood flow are measured in the same brain slices (4).

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Several procedures for labelling of 4-iodoantipyrine with radioactive iodine have been reported. In general two routes can be followed. One is the exchange reaction between radioactive iodine and 4-iodoantipyrine or 4-bromoantipyrine (5). The second route is direct introduction of radioactive iodine into antipyrine (6). The former procedure has been favoured because of its good yields and because there has been no real need for preparation of a carrier free or high specific activity 4-iodoantipyrine.

Recently Boothe et al used direct introduction of radioactive iodine to prepare labelled 4-iodoantipyrine by reacting antipyrine with radioactive iodine on thin layer chromatographic (TLC) plates (6). Although the procedure they describe produces a good yield, it is not practical for regular preparation of iodine labelled 4-IAP because the TLC plate must be scratched to recover the labelled material.

In this paper we describe a simple procedure for the preparation of carrier free ("no carrier added") 4-iodoantipyrine. The method uses a technique of dry column chromatography which effectively separates various materials (7).

MATERIALS AND METHODS

In our work we have used a carrier-free ("no carrier added") solution of $Na^{131}I$ commercially obtained †. The solution of $Na^{131}I$ in water contained no stabilizer or preservative. The isotope was supplied in a total volume of about 50 µl (having 100 mCi/ml).

† Merck Frosst Inc., Montreal, Quebec

To find optimum conditions for the separation of 4-IAP (2) and antipyrine (1), which could be directly transferred to dry column chromatography, we carried out a series on the TLC plates made from the same material* later used in dry columns. In our search for the best solvent mixture we had two requirements: good separation of 4-IAP and antipyrine, and an R_f value of 4-IAP above 0.85 to ensure elution with the front of the solvent. These requirements were fulfilled by using toluene-ethyl acetate-ethyl alcohol (1:2:3) as a developing solvent. R_f values for 4-IAP and antipyrine in that solvent are 0.92 and 0.67, respectively.

Antipyrine used in our work was of high chemical purity††with only one spot on TLC plates in two different solvents. The quality control in our work was done on non-activated hard layer 250 μm thick silica gel TLC plates**. TLC plates were developed in a saturated chamber and the plates examined under an ultraviolet light at 254 and 360 nm. These plates with radioactive iodine as a tracer were scanned on a radiochromatograph with a windowless proportional counter equipped with a 3 mm opening. 4-iodoantipyrine used as an internal standard for TLC was obtained commercially***.

Silica gel used for dry column chromatography †††was the same as that used on the TLC plates and it was used without any activation. The column used in this work had a 0.9 cm internal diameter; in general silica gel was packed in the column to height of about 6 cm. The solvent mixture used in the development and in situ labelling was toluene-ethyl acetate-ethyl alcohol (1:2:3).

* Polygram Silica Gel N-HR/UV 254, Sybron/Brinkmann
** Cat. No. AN47521, Silica Gel HLF, analtech
*** Chemicals Precurement Laboratories, Inc., College Point, NY.
†† Cat. No. A-5882, Sigma Chem. Co.
††† Silica Gel N-HR/UV 254, Sybron/Brinkmann

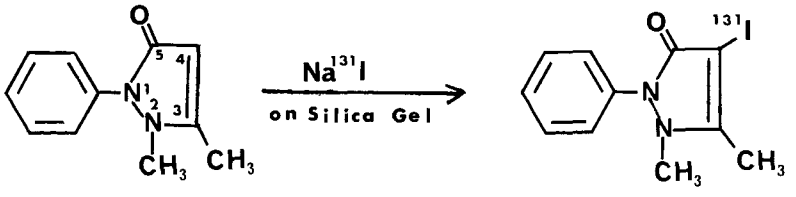
To the top of the column 10 μl of $Na^{131}I$-solution was added along with 4 mg of antipyrine and 5-10 μl of 5N hydrochloric acid. A control mixture was put in a sample vial, without organic solvent and silica gel, and the vial was tightly closed. After five minutes approximately 100 mg of silver oxide or silver carbonate and one ml of distilled water were added and the solid was removed by filtration through a millipore membrane. The radioactivity carried with silver precipitate was assumed to be inorganic iodine. The labelling of iodoantipyrine occured in the control vial to the extent of up to 40 per cent. There was no special care to exclude oxygen from the control mixture. The labelling yield is increased to 80 per cent on average by loading the mixture on a dry silica gel column and eluting (^{131}I)-4-iodoantipyrine with a proper mixture of solvents. To prove that labelling is specific to carrier free iodine, we experimented by adding small amounts of inactive NaI. Addition of even 1 μg of NaI into the reaction mixture stopped the labelling reaction in the control vial. Putting this reaction mixture through a silica gel column, as described earlier, increased the labelling yield to 6 per cent.

To find optimum labelling condition the acid concentration was varied and the best labelling yield was achieved by adding 5N hydrochloric acid. Material was fixed on the top of the silica gel with a small amount of glass wool and the developing solution slowly applied to the top of the column. The supply of solvent is carefully controlled to prevent high hydrostatic pressure from the top. As mentioned earlier, the solvent mixture for dry column chromatography was optimised by TLC. If one wants to prepare several columns it is easier to make up the mixture of $Na^{131}I$, antipyrine, HCl and silica gel first and then to put the appropriate amount at the top of each column.

RESULTS AND DISCUSSION

One tenth of a milliliter fractions of the eluting solvent were collected and analysed on TLC plates. We found that (^{131}I)-4-IAP starts to elute in the third fraction. The entire iodoantipyrine elutes in about 0.6 ml of solvent.

The labelling procedures yield was measured by comparing the activity of the Na^{131}I added to the column with that of (^{131}I)-4-IAP (2). The labelling reaction can be represented as follows:



(1) (2)

(^{131}I)-4-IAP was assayed by TLC in two different solvents. In both solvents $^{131}I^-$ would stay at the point of origin whereas (^{131}I)-iodine would move to the front. Radiochemical purity of (^{131}I)-4-IAP exceeds 98 per cent. A TLC radiochromatogram of the final product is given in Fig. 1. A small amount of antipyrine (1) and inorganic I^o (less than 1 per cent) is present in the final (^{131}I)-4-IAP. Inorganic I_2 is most likely produced by radiolyses of Na^{131}I. Trace amounts of antipyrine impurity can be removed if desired, by passing the final product through a second column of silica gel and using less polar solvent. Here it should be noted that a few micrograms of antipyrine should not effect measurement of the rCBF with (^{131}I)-4-IAP prepared by this procedure and it is therefore not necessary to remove it from (^{131}I)-4-IAP. Inorganic iodine can be removed by

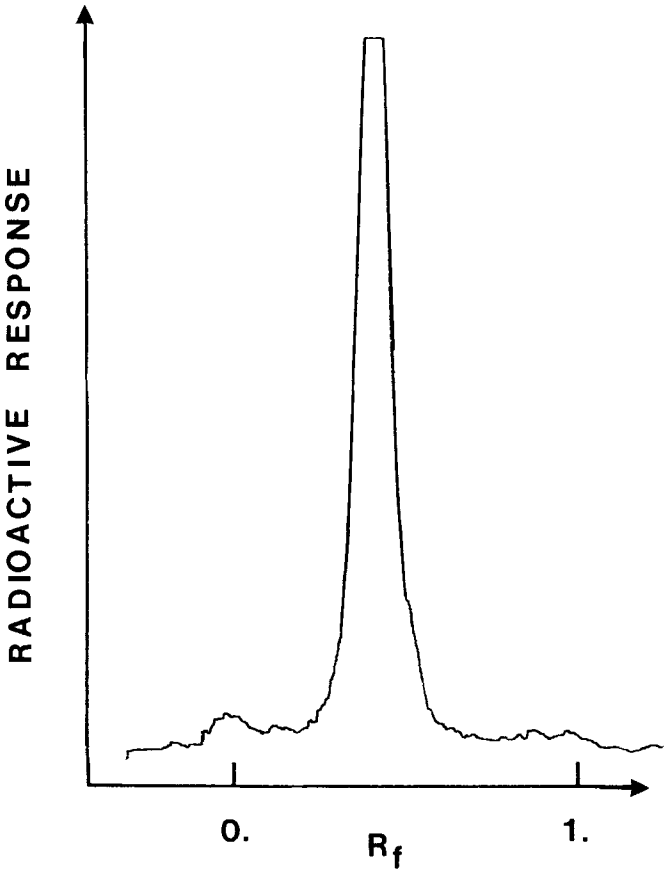
adding a small amount (about 3 mg) of Ag_2O and filtering it. An additional test for the presence of iodine and iodide was carried out. Into the organic layer was added 200 µg of 4-IAP and 3mg of Ag_2O. Silver oxide was filtered off and washed with organic solvent to remove all traces of (^{131}I)-4-IAP. The radioactivity on silver oxide, was assessed with an NaI(Tl) detector connected to a single channel analyser, was found to be less than 2 per cent. From experiments with silver oxide and TLC-radiochromatograms, we have concluded that there was very little ionic or atomic iodine present in our 4-IAP, since both of them would precipitate as AgI.

Our procedure, which can be used with any iodine radioisotope, produces around 80 per cent carrier free (^{131}I)-4-IAP. Stability of carrier free (^{131}I)-4-IAP was assessed by storing it in a refrigerator in a methanol or 0.9 per cent saline solution. Not more than 1 per cent decomposition was noticed in either solvent after thirty days when total activity in the vial was less than 1 mCi. Similar results were observed by DiMattio and Hochwald (3).

On TLC plates, (^{131}I)-4-IAP prepared by the method above, has the same R_f-value as inactive commercially obtained 4-IAP which was visible under UV-light at 254 nm. Thin layer chromatograms were developed in two different solvent mixtures, ethyl acetate-toluene (1:1) and toluene-ethyl acetate-chloroform (2:1:1). R_f values observed in these solvents were 0.38 for the first one and 0.64 for the second.

Although the labelling procedure described by Robinson and Lee (5) might be effective, the labelling yield is not as high as claimed and the final elution through anion resin to remove radioiodine does not work in our hands as described in the paper (5). Accordingly we have tried to prepare ^{131}I-labelled 4-IAP by the

Figure 1: TLC Radio-chromatogram of Carrier Free ^{131}I -4-IAP, developed in ethyl acetate-toluene (1:1).



Robinson-Lee method with the following change: we measured the labelling yield and purity after removing whatever I^- and I_2 might be present by precipitating AgI after adding 10 mg of I^- carrier. The yields were around 50 per cent. This precaution avoids erroneous conclusions that might be made from the assessment of the purity and yield of labelled 4-IAP by TLC on silica gel, since the labelling of antipyrine might occur in a high yield on TLC plate (6).

In experiments where (^{131}I)-4-IAP prepared by the method described in ref. 5 was eluted with 0.9 per cent NaCl, most of the(^{131}I)-4-IAP was not eluted from ion exchange resin in the first 1 ml of eluent as stated by the authors (5). In our hands at least it was eluted in quite a large volume. Furthermore, we found that the elution works much better with methanol than with 0.9 per cent saline solution.

Labelling on TLC plates (6), even if it results in a satisfactory yield, is not practical since it requires scraping of the spot. This is not really advisable with radioiodine in everyday preparations, or with any other radioisotopes.

The mechanism of incorporation of iodine into the molecule of antipyrine is not completely clear. Iodoantipyrine is classically produced through iodination of antipyrine with iodine (3), produced <u>in situ</u> by reaction of KI and KIO_3. In this reaction there is definitely a transit state of I^+ which some researchers (8) believe is needed for the incorporation into the antipyrine molecule. If this mechanism does occur in the reaction it might also explain our results. The presence of I^+ in our reaction mixture is not in doubt, because the solution used in our work is carrier free and the presence of this species (I^+) as an intermediary oxidation state, produced by radiolyses in radioiodine solutions. Since labelling was increased after passing the mixture through a silica column, it is clear that the surface effect

plays a very important role in this labelling mechanism. If assumed that I^+ is needed for labelling it seems that I^+ must be produced by the interaction of I^- or I° with the silica gel surface because passing of the reaction mixture throughout the column increases labelling yield. The labelling on TLC plates as described in reference 7 is a surprise, because the authors state that their radioactive iodine had $NaHSO_3$ as a stabilizer. In the presence of a reducing agent, $NaHSO_3$, the existence of I^+ would be highly unlikely. It is possible, however, that $NaHSO_3$ is decomposed on the TLC plate. It is also possible that after the spot dries, the effect on iodine of a reducing agent in an organic solvent becomes negligible. It is not surprising that Boothe et al (6) did not observe any labelling before applying their mixture to silica gel plates (in control vials), because their iodine had $NaHSO_3$ as a stabilizer in the ^{131}I solution.

CONCLUSION

A simple labelling procedure using dry column chromatography produces carrier free ^{131}I-labelled 4-iodo-antipyrine in an average radiochemical yield of 80 per cent (the best being 90 per cent). The radiochemical purity of(^{131}I)-4-IAP exceeds 98 per cent. The method does not require any special skill and could be set up and done on a routine basis without any special technical attention. This method could be of use in a variety of set-ups, for instance columns could be prepared in an institution or in an isotope producing laboratory and shipped to the user's laboratories, and developed there to produce labelled 4-IAP.

It has been shown that labelling of iodoantipyrine occurs to the extent of 40 per cent by the simple reaction between species of carrier free iodine and antipyrine in a glass vial. The labelling yield has been increased by passing the mixture through a silica column has been demonstrated.

Since characteristics of commercial silica gel vary from supplier to supplier one should use the same silica gel we used with our solvent mixture if the same results are desired. If another silica gel is used one should measure R_f values before attempting to label 4-iodoantipyrine.

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