# Kit Method of Preparation of 4-Iodoantipyrine (I-123) from $Na^{123}I$: Concise Communication

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***4-Iodoantipyrine (4-IAP), containing $^{123}I$, has been suggested as a radiopharmaceutical for direct imaging of regional cerebral perfusion. In routine clinical use, a reliable source of the labeled compound is required. The 4-IAP (I-123) can be prepared by exchange between $Na^{123}I$ and 4-bromoantipyrine (4-BrAP) or 4-IAP with heating in aqueous acidic solution. Using an $H_3PO_4$ buffer system at pH 2.5 during labeling, with addition of NaOH for subsequent buffering at pH 7.0, a convenient reliable kit for routine preparation of 4-IAP (I-123) from commercially available $Na^{123}I$ has been developed.***

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4-Iodoantipyrine* (4-IAP) is a relatively lipophilic molecule that readily diffuses across cell membranes (*1–4*). Immediately after intravenous injection, levels of radioiodinated 4-IAP within brain tissue are higher than those for ionic tracers such as $^{42}K$ (*5*). This has been attributed to absence of a blood–brain barrier for 4-IAP.

4-Iodoantipyrine (I-131) has been used to measure relative regional cerebral blood flow in animals and man (*5–7*). Uszler et al. studied the feasibility of directly assessing human cerebral perfusion using 4-IAP (I-123) and the scintillation camera (*8,9*). For use in humans, $^{123}I$ was the label of choice because of its superior physical characteristics with respect to camera imaging and because of the millicurie doses that can be safely administered.

Extension of this technique to the routine clinical setting depends on a convenient reliable source of 4-IAP (I-123). We have recently reinvestigated the influence of pH and reactant concentrations on preparation of 4-IAP (I-131) by exchange between the iodide and unlabeled 4-IAP or 4-BrAP (*10*). As a result of our findings, and considering the commercial availability of $Na^{123}I$, we have developed a simple reliable kit method for routine preparation of sterile pyrogen-free 4-IAP (I-123) (*11*). The kit has been used successfully for preparation of 4-IAP (I-123) used in preliminary clinical studies (*8,9*).

## MATERIALS AND METHODS

Our studies on the synthesis of 4-IAP (I-131) from $Na^{131}I$ used 4 mg of 4-IAP, or 10 mg of 4-BrAP, with heating at acid pH in a boiling water bath. Optimal yields were obtained within 5 min. In the pH range between 2 and 6, quantitative yields of the labeled product were achieved if 4-IAP was used as the unlabeled starting material. When 4-BrAP was used, little labeling occurred above pH 3, and quantitative yields were never produced. Maximum labeling (~ 80%) occurred only in the pH range between 1.5 and 2.5. The free radioiodide was removed by anion exchange.

The ultimate purpose for our $^{131}I$ studies was to develop a method for routine production of 4-IAP (I-123) for clinical use as an imaging agent for cerebral perfusion. To simplify the synthesis as much as possible, a simple kit method for preparing 4-IAP (I-123) from commercially available $Na^{123}I$ was developed. Specifications for preparation of the necessary components for 20 such kits are as follows:†

1. *4-Iodoantipyrine reagent:* Dissolve 80 mg

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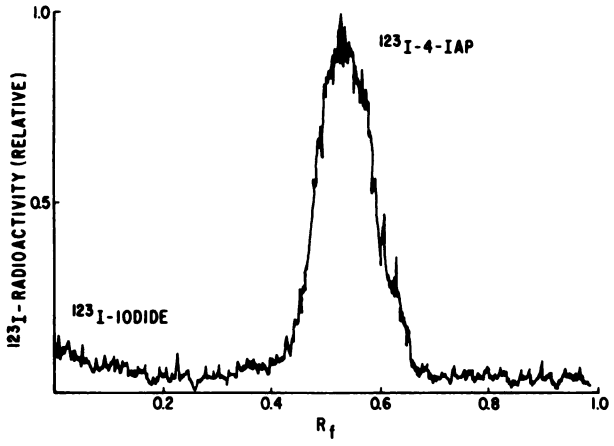


**FIG. 1.** TLC analysis showing radiochemical purity of 4-IAP (I-123) prepared by kit method. $Na^{123}I$ appears near origin, and 4-IAP (I-123) at $R_f = 0.55$.

of 4-IAP‡ or 200 mg of 4-BrAP|| in 20 ml of distilled water (*USP*). Heating may be required to dissolve the 4-IAP. Dispense 1-ml aliquots of this "labeling reagent" into sterile 10-ml serum vials. The solution may be lyophilized before the vials are capped.

2. *0.1* M *phosphoric acid:* Dilute 0.25 ml of 85% $H_3PO_4$ (ACS reagent) to 21.5 ml with distilled water (*USP*). Dispense 1-ml aliquots into sterile 2-ml serum vials.
3. *0.5* N *sodium hydroxide:* Dissolve 500 mg of NaOH (ACS reagent) in 25 ml of distilled water (*USP*). Dispense 1-ml aliquots into sterile 2-ml serum vials.
4. *Anion exchange column:* If 4-BrAP is used for kit preparations, free radioiodide can be removed from the labeled 4-IAP by anion exchange. Place a glass-wool plug in the end of a 3-ml disposable syringe barrel. Fill to the 1-ml mark with Dowex 1-X8 anion exchange resin (chloride form, 50–100 mesh) and secure it with a second glass-wool plug on top of the column. If this procedure is used, terminal sterilization of the product is required.

Using these kits, 4-IAP (I-123) is rapidly and efficiently prepared by chemical exchange with $^{123}I^-$ at pH 2.5. When $H_3PO_4$ is used for acidification, subsequent neutralization with NaOH gives an injectable buffered solution at pH 7. The procedure for labeling is as follows:

1. The "labeling vial" contains 4 mg of 4-IAP or 10 mg of 4-BrAP.
2. Add a 1–4-ml volume of the desired activity of $^{123}I^-$ (pH 4–10) to the labeling vial.
3. Acidify the labeling mixture by adding 1 ml of 0.1 *M* $H_3PO_4$.
4. Immerse the labeling vial in boiling water for 5 min and allow it to cool.
5. Neutralize and buffer (pH 7) the acidified labeling solution by adding 0.5 ml of 0.5 *N* NaOH.
6. Free $^{123}I^-$ can be removed by passing the buffered product through an anion exchange column eluted with 0.9% NaCl.

If the radioiodide is supplied in dilute NaOH solution, simple addition of the appropriate quantity of HCl will achieve approximately the required neutralization (pH 4–10). When dilute $NaHCO_3$ solution is supplied, adding the acid results in evolution of $CO_2$ gas. In this case, venting of the vial during addition of HCl, and subsequent incubation of the neutralized $^{123}I^-$ solution at 80°C for 10 min, frees the solution of dissolved $CO_2$ so that a pressure buildup during labeling is avoided.

In clinical use the 4-IAP (I-123) is terminally sterilized by membrane filtration (0.22 $\mu$m), although all containers except anion exchange columns are presterilized and aseptic technique is used throughout the labeling procedure.

## RESULTS AND DISCUSSION

When the kit method is used to prepare 4-IAP (I-123) for routine clinical use, the radiochemical purity of the final product exceeds 95%, as measured by thin-layer chromatography (TLC) on silica gel using 1:1 (vol/vol) ethyl acetate–toluene as the solvent. With this analytical system, iodide remains near the origin while 4-IAP migrates with an $R_f$ value of 0.55. The TLC analysis of a typical 4-IAP (I-123) kit preparation is shown in Fig. 1, and the results from four successive preparations are summarized in Table 1.

Radioiodide solutions are usually obtained in dilute aqueous base (*12–15*) and neutralization of this base is required before acidification for labeling.

**TABLE 1. RADIOCHEMICAL PURITY OF FOUR CONSECUTIVE KIT PREPARATIONS OF 4-IODOANTIPYRINE (I-123)**

| Date | Activity (mCi) | 4-IAP (I-123) (%) | $Na^{123}I$ (%) |
|---|---|---|---|
| 7–9–75 | 15.6 | 96.3 | 3.6 |
| 7–23–75 | 12.3 | 97.2 | 2.9 |
| 8–20–75 | 17.7 | 94.8 | 5.2 |
| 9–10–75* | 11.7 | 98.3 | 1.7 |

* 4-BrAP used for labeling. $Na^{123}I$ removed by anion exchange. Actual radiochemical yield of 4-IAP (I-123) was 91.2%.

While precise control of labeling pH is not required if 4-IAP is used, the reliability of kit preparations is improved if the reaction occurs under reproducible conditions. If 4-BrAP is used, precise pH control during labeling is essential. In order to eliminate the necessity of an acid–base titration—or using a pH meter or pH paper—to obtain precise pH control during acidification, we selected an $H_3PO_4$–NaOH buffer system. If 1–4 ml of $^{123}I^-$ in dilute base is brought to a pH between 4 and 10 by approximate neutralization with a small volume of HCl, adding 1 ml of 0.1 *M* $H_3PO_4$ gives a buffered reaction solution in the pH range between 2.3 and 2.5. This is because the dilute $H_3PO_4$ solution, with $K_1 = 7.5 \times 10^{-3}$, provides buffering in this pH range. After this acidification and subsequent heating, cooling, and venting of the reaction vial, adding 0.5 ml of 0.5 *N* NaOH converts the reaction mixture to a buffered solution at pH 7. Using this buffer system, precise reaction and final pH control are obtained, but no direct pH measurements are required. The kits have been found to be compatible with $^{123}I$ supplied as iodide by UC Davis, UCLA, and Medi-Physics. In the latter case, $^{123}I^-$ is supplied in dilute $NaHCO_3$ and the described precautions for neutralizing such solutions were used. These kits have been used for routine clinical preparation of 4-IAP (I-123) with complete reliability (*8,9*).

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FOOTNOTES

* 4-Iodo-1,5-dimethyl-2-phenyl-3-pyrazolone.

† All solutions are sterilized by membrane filtration (0.22 μm) during dispensing and aseptic technique is used throughout.

‡ American Felsol.

|| Pfaltz and Bauer, Flushing, N.Y.

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