KIT TYPE LABELLING OF [^{123}I] METAIODOBENZYLGUANIDINE

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SUMMARY

We have examined the labelling of [^{123}I] MIBG by a Cu(I) catalyzed reaction in aqueous solution. We regularly obtained >99% radiochemical purity [^{123}I] MIBG product and >97% radiochemical yield. At 100°C with 2 mg MIBG the radiochemical purity reached 85% at 2.5 min, 97% at 5 min and 99% at 10 min through 30 min. With 200 μg MIBG the reaction proceeded at roughly one tenth the former speed. Many details of technique were essential; the highlights were use of dry triturated reagents and the use of H_2SO_4 solvent. Neglect of proper procedure led to low radiochemical yields and ^{123}I bound to apparatus. The procedure has been utilized with up to 1.0 mL ^{123}I (0.1N NaOH) or 93 mCi ^{123}I. A study of temperature dependence showed that the reaction proceeded readily, albeit more slowly at 20°C. The temperature coefficient at 100°C was estimated ~1.7.

Key words: Metaiodobenzylguanidine, adrenergic blocker, radiolabelling, ^{123}I.

INTRODUCTION

Radioiodide exchange labelling is presently an essential procedure used in the clinic to prepare radiopharmaceuticals. The ideal procedure for labelling would be convenient and give rapid and quantitative incorporation of radioiodide into a pharmaceutically acceptable product. In practice, given a valuable radiopharmaceutical, one will usually find that the available means of labelling are unsatisfactory in one or several respects. A baffling aspect is that there is a high molar ratio of pharmaceutical precursor to no carrier added iodide in many exchange reactions. The molar ratio may approach one million to one. Yet the radioiodide exchange is typically significantly less than 100% when the labelling conditions have been optimized. Small molar quantities of radioiodine have hindered analyzing why such limitations exist.

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The pharmaceutical, meta iodobenzyl guanidine (MIBG)[1], has proven valuable in several different clinical applications and promises to be in increasing demand. MIBG is an analog of the adrenergic neuron blocking drug guanethidine and is accumulated by adrenergic cells. This characteristic allows MIBG to usefully label a variety of organs and tissues including adrenal medulla[2], heart[3], pheochromocytoma[4], and neuroblastoma[5]. Current research includes protracer derivatives potentially applicable to brain imaging[6]. [^{123}I] MIBG offers advantages over the ^{131}I product, including absence of β decay, shorter decay half life, and applicability to SPECT.

Recent research has emphasized the importance of MIBG kits[7], since conventional preparations[8,9] require at least 1.5 hours at 150°C to achieve exchange labelling. For this reason innovative "kit form" analogs of MIBG were proposed because they are suited to oxidative labelling requiring only 15 min. In the analogs, amino or hydroxy groups have been introduced to activate the aromatic ring towards electrophilic iodination in a kit procedure[7]. Preliminary observations have emerged that the "kit form" analogs [4-amino-3- iodobenzylguanidine (AIBG) and 4-hydroxy-3-iodobenzylguanidine (HIBG)] may offer valuable physiological differences from MIBG[7]. Despite these advances, an MIBG kit is needed. Tracer biodistribution criteria, not labelling behaviour, should ideally determine which MIBG analog is optimum.

An alternative exists which deserves wider recognition and use in clinics. This is the Cu(I) mediated labelling of aryl iodide compounds developed by Mertens and his colleagues [10-14]. In this approach a transient _in situ_ copper-organo complex is formed and is labelled to >99% in several examples. Systematic investigations were originally carried out using 4-iodo-N-isopropyl amphetamine (IMP)[11-13]. The results proved to have more general applicability to a variety of aryl iodide radiopharmaceuticals including o-iodohippuric acid[12], MIBG[12], N,N,N-Trimethyl-N-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine sulfate (HIPDM)[14], various iodophenyl fatty acids[15-16], and p-iodospiperone[14].

The purpose of this investigation is to outline a useful procedure for quantitative kit type labelling of MIBG, to evaluate the kinetic performance, and to elucidate pitfalls in the procedure.

MATERIALS AND METHODS

The HPLC system utilized a C18 reverse phase column (25 cm × 3.9 mm) and a UV detector operating at 254 nm. ^{123}I was measured in a well type NaI detector or in a dosimeter (Capintec). Labelling at 100°C was carried out in 5 mL borosilicate serum vials with uncoated red rubber septa (Wheaton 223738 and 224124). Filters were Millipore 0.22 μm (SLGS 0250S). Temperatures were measured using a 33 gauge × 1 in copper-constantan thermocouple with monitor.

[^{123}I]NaI supplied in 0.1 N NaOH (0.3 to 0.5 mCi/μL) was produced via the ^{124}Xe(p,2n)^{123}I reaction (Atomic Energy of Canada Ltd.). Isotopic purity was ^{123}I > 99.8%, iodide >99.0%. MIBG sulphate (Edmonton Radiopharmaceutical Centre): mp 164-165°C. HPLC[12] (methanol/water/acetic acid/trimethyl amine 57/53/0.5/0.5 1.0 mL/min) showed only one peak, t_R = 7.2 min. IR(KBr) 3420 and 3160 (NH), 1670 and 1630 (C=N), 1120 (S=O), 770 and 690 cm^{-1} (1,3 disubstituted benzene). PMR (CD_3OD) δ4.16 (S,2,CH_2), δ6.9-7.7 (m(seven peaks), 4, arom). Anal: calcd. for $C_8H_{10}IN_3(0.5H_2SO_4)$: C,29.65, H,3.42, N,12.96; found C,29.39, H,3.44, N,12.71. TLC performed on silica gel plates for 30 min utilized ethanol/ammonium hydroxide in the ratio 3/1[8]. Rf values were [^{123}I] MIBG, 0.15, [^{123}I]NaI, 0.90.

<u>^{123}I Labelling of MIBG</u>: Three reagents were first prepared: the dry kit, H_2SO_4, and ^{123}I.

1. <u>Dry Kit</u>: consisted of MIBG sulphate (2 mg) plus reductant (10 mg). Excess reductant was first made using mortar and pestle in a nitrogen filled (N.F. 30 ppm oxygen) glove box. Static electricity was controlled with a piezoelectric ionizer (Zerostat). A specific order was followed in pulverizing, namely $CuSO_4 \cdot 5H_2O$ (30 mg) followed by ascorbic acid (2.0 g) followed by $SnSO_4$ (95+%, Aldrich) (100 mg). Thirty 12 mg kits were dispensed (and sealed) in the

nitrogen atmosphere from a mixture of 60 mg MIBG sulphate and 300 mg reductant. Thus the quantity per vial was: MIBG, 2 mg, ascorbic acid, 9.4 mg, $SnSO_4$, 470 μg and $CuSO_4 \cdot 5H_2O$, 140 μg. Kits were subsequently stored up to six months at 5°C before use. Alternative kit formulations were made using a similar approach. (A more convenient preparation was in open air followed by nitrogen purging of solids in sealed vials. In this case the shelf life was only one week or so, when discolouration of the solids became evident.)

2. $\underline{H_2SO_4}$: Once distilled water (with 10 μL 1 M H_2SO_4 added per mL) was sealed in a serum vial and flushed under nitrogen gas for a total of 30 min.

3. $\underline{^{123}I}$: 100 μL distilled water was added followed by an appropriate volume of ^{123}I. The vial was then sealed and flushed 5 min under nitrogen gas.

Labelling Procedure: Operations were carried out in a radioiodination hood. A 2 in × 25 gauge hypodermic needle was attached to a 1 mL tuberculin syringe. With air tight technique 0.9 mL stock H_2SO_4 was drawn into the syringe, and this was added to the dry kit. With the hypodermic needle in place, the solids vial was gently rocked for about 30 sec while the reagents dissolved. The solution was drawn into the syringe and injected gently into the [^{123}I] NaI stock. This final mixture was flushed with nitrogen for approximately 2 min. The reaction mixture was heated a stated time in a covered boiling water bath. Samples intended for kinetic analysis were subsequently chilled for 2 min in an ice bath. The product was drawn up into a syringe and filtered (0.22 μm filter) into a final nitrogen purged vial. A wash of the reaction vial was carried out with 1 mL water which was likewise filtered into the final vial. A 10 μL sample was withdrawn into 1 mL methanol for quality evaluation. (Products for human injection utilized the same operations but different solutions: Immediately following labelling 0.4 mL 0.4 M phosphate buffer pH 7.0 (USP) was added to the [^{123}I] MIBG solution prior to filtration. The labelling vial was

subsequently washed with 1 mL saline (USP). The pH of the final product was approximately 6.8.)

RESULTS

Two significant problems were identified affecting the labelling of MIBG. Initial experiments showed there is a need for dry anaerobic reagents. Our original technique consisted of combining suitable portions of reagent solutions (i.e. ascorbic acid, $CuSO_4$, $SnSO_4$ and ^{123}I) followed by nitrogen flushing and heating. This procedure invariably led to white precipitate, ^{123}I "bound" to vial and filter (i.e. "bound" ^{123}I could not be washed from the labelling vial or filter by water or saline), yellow solution and rather low radiochemical purity in the product (Table 1A, 1.1 to 1.4). A possible explanation is that Sn(II) was quickly converted to Sn(IV) in aqueous solution. The problem was largely overcome by the use of dry triturated reagents in the absence of oxygen (Table 1B 1.5).

Need for control of pH was made evident when it was noted that volumes of ^{123}I greater than ~80 μL brought about irregular behavior, i.e. white precipitate, "bound" ^{123}I, and low radiochemical purity of the [^{123}I] MIBG. For example in one case with 100 μL ^{123}I 99.4% radiochemical purity was obtained while 18% of ^{123}I was bound to the vial and filter. In a second example with 170 μL ^{123}I 82% radiochemical purity was obtained in the product solution while 5% of the ^{123}I was "bound". The problem was attributed to hydroxide ion accompanying the ^{123}I. The problem was controlled by carrying out the labelling reaction in H_2SO_4 (0.01 M) with additional H_2SO_4 as necessary to acidify volumes >80 μL of ^{123}I solution. In a control experiment we attempted to label MIBG in the presence of H_2SO_4 alone ($CuSO_4$, $SnSO_4$ and ascorbic acid were omitted); no labelling was obtained in this case.

The ^{123}I bound to serum vial and filter is thought to be [^{123}I]CuI (solubility product ~$5\times10^{-12}M^2$). Two preliminary hypotheses concerning the "bound" ^{123}I were not supported. Conspicuous white precipitate accompanying the "bound" ^{123}I prompted the hypothesis that ^{123}I was

Table 1

[^{123}I] MIBG labelling with aqueous and dry reagents

Parameters*:

Comment:		MIBG	$SnSO_4$	Solvent	Temp.	Radiochemical Purity [^{123}I]MIBG(%)	Binding Loss Filter	Binding Loss Vial
Aqueous reagents								
A	1.1	1.0 mg	500 µg	300 µL H_2O	100°C	94.6%(63.3%)†	30%	3%
	1.2	1.0 mg	0	300 µL H_2O	100°C	96.1%(42.2%)	25%	31%
	1.3	1.0 mg	0	300 µL H_2O	140°C	97.0%(64.0%)	20%	14%
	1.4	100 µg	0	300 µL H_2O	140°C	95.7%(86.1%)	6%	4%
	Dry reagents							
B	1.5	2.0 mg	500 µg	1.0 mL H_2O	100°C	98.3%(93.6%)	3.1%	1.7%
	1.6	2.0 mg	500 µg	1.0 mL H_2SO_4	100°C	99.5%(98.2%)	0.8%	0.5%
	1.7	2.0 mg	0	1.0 mL H_2SO_4	100°C	90.3%(81.4%)	2.8%	7.0%

*Common parameters include $CuSO_4 \cdot 5H_2O$ (150 µg), ascorbic acid (10 mg), [^{123}I] NaI (5 mCi) and time of heating (30 min). When used, H_2SO_4 was 0.01 M.

†Radiochemical Yield of [^{123}I] MIBG: Radiochemical purity × (100% - Binding Loss), e.g. 94.6% × (100% - 33%) = 63.3% in example A1.1.

bound in a tin compound. Alternatively, the abundance of [^{123}I] MIBG in solution suggested that the bound ^{123}I might exist largely in the form of [^{123}I] MIBG. Binding of ^{123}I was frequently found in the absence of $SnSO_4$ (e.g. Table 1A, 1.2); this suggested that the bound ^{123}I was not in a tin compound or precipitate. The possibility of bound [^{123}I] MIBG was disproved when standard kits were made up in the presence of air or with added NaOH (300 µL of 0.1 N NaOH). Bound ^{123}I was obtained, 28% in the presence of air or 25% with added NaOH. The product radiochemical purities measured 80% and 75% [^{123}I] MIBG. The bound ^{123}I was dissolved

in 5% aqueous NH_4OH and analyzed by TLC (Materials and Methods) where it migrated as iodide (>97%). In a second experiment, $CuSO_4$, ascorbic acid and [^{123}I]NaI in water, proved to be sufficient and necessary reagents to generate 98% ^{123}I binding in 5 min at 100°C.

Acetic acid was evaluated as an alternative solvent since there has been interest shown in HAc in the recent literature.[9,11,13] Using 70% HAc with no $SnSO_4$ at 140°C greater than 95% labelling was obtained (Table 2) while the ^{123}I bound was less than 4%. While acetic acid might be seriously considered as a solvent it had the disadvantage that half the ^{123}I activity was lost as vapor during two minutes of nitrogen flushing. By comparison some 0.5% of the ^{123}I was lost during flushing of stock H_2SO_4 as measured utilizing activated charcoal traps.

Labelling was measured at 100°C using 2 mg MIBG per sample. The radiochemical purity results in Table 3 and Fig. 1 show that labelling commenced rapidly without a lag, and attained a value of 85% by 2.5 min.

Table 2

[^{123}I] MIBG labelling in acetic acid

MIBG*	$SnSO_4$	Temperature	Radiochemical Purity [^{123}I]MIBG(%)	Binding Loss Filter	Binding Loss Vial
1.0 mg	500 µg	100°C	96.4%(77.1%)†	4%	16%
1.0 mg	0	140°C	93.3%(74.6%)	7%	13%
100 µg	500 µg	140°C	95.4%(92.7%)	1.4%	1.4%
100 µg	0	140°C	96.4%(92.9%)	1.8%	1.8%

*Common Parameters: $CuSO_4 \cdot 5H_2O$ (150 µg), ascorbic acid (10 mg), 70% acetic acid (30% H_2O) (300 µL), ^{123}I[NaI], and time of heating 30 min.

†Radiochemical yield, not including volatile ^{123}I lost.

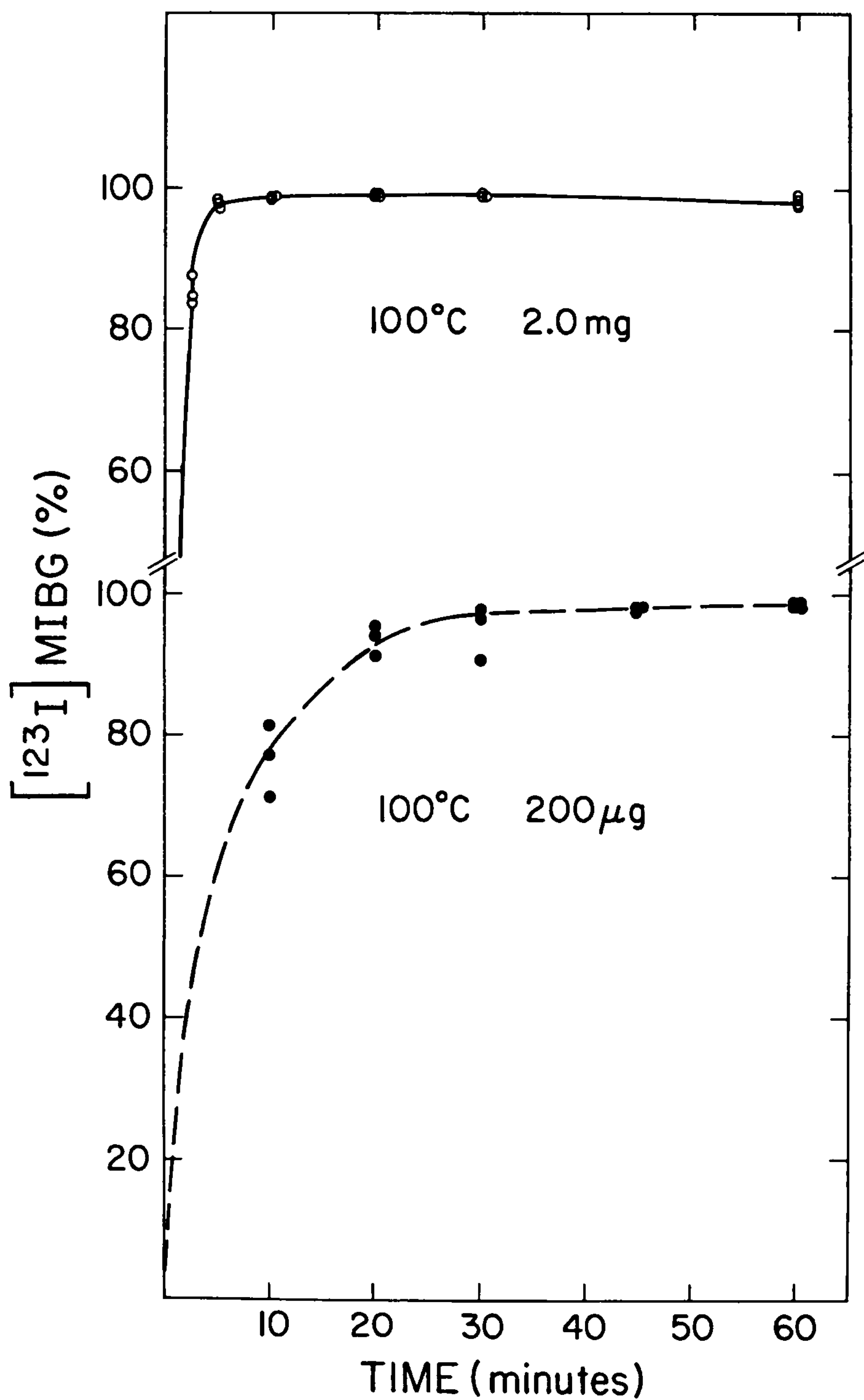

Fig. 1. Radiochemical purity of [^{123}I]MIBG product at 100°C with 200 µg (●) or 2.0 mg (o) MIBG per reaction mixture.

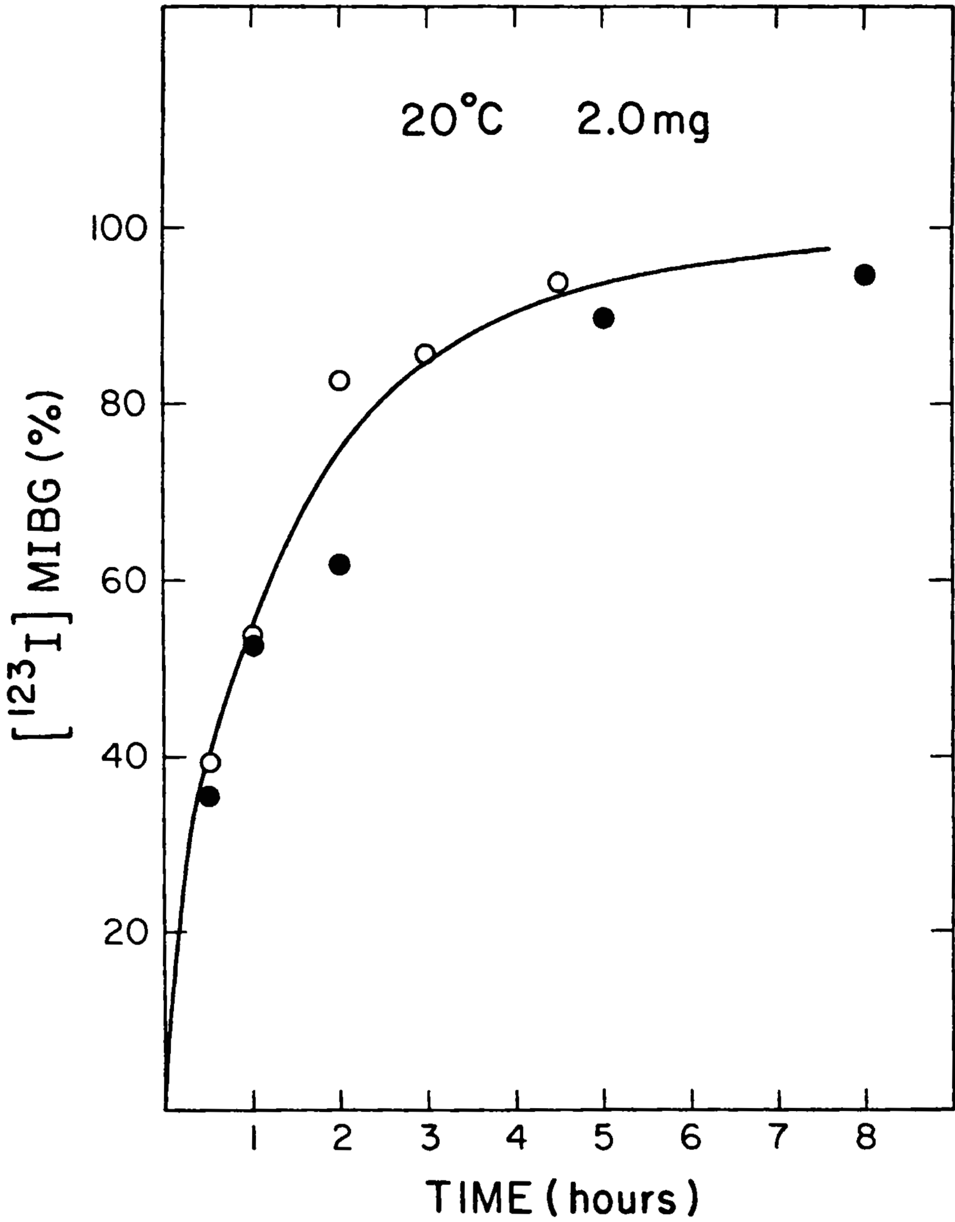

Fig. 2. Radiochemical purity of [^{123}I]MIBG product at 20°C with 2.0 mg MIBG per reaction mixture in two trials [(●) and (o)].

Table 3

Radiochemical purity of $[^{123}I]$MIBG (100°C)

Time (min)	2 mg kit $[^{123}I]$MIBG %(n)	200 μg kit $[^{123}I]$MIBG %(n)
2.5	85.4 ± 2.1%(3)	---
5	97.6 ± 0.5%(3)	---
10	98.9 ± 0.1%(3)	76.7 ± 5.0%(3)
20	99.3 ± 0.2%(4)	93.6 ± 2.0%(3)
30	99.4 ± 0.1%(3)	95.0 ± 3.7%(3)
45	---	98.4 ± 0.2%(3)
60	98.7 ± 0.7%(4)	98.9 ± 0.3%(4)

*Radiochemical purity was determined by HPLC: $[^{123}I]$MIBG × 100% / $\left([^{123}I]\text{MIBG} + [^{123}I]\text{ iodide}\right)$.

It is clear that a useful product (~97.5% radiochemical purity) was obtained in as little at 5 min. This is definitely a shorter time than previously reported in the literature. The maximum radiochemical purity was attained at 20 to 30 min, where the mean was 99.4 ± 0.2% (n=7). To a crude approximation the radiochemical purity was asymptotic to 100%, although in fact the value at 60 min was reduced to 98.7%. The data include both 10 mCi and 20 mCi ^{123}I per sample, with no obvious difference. The ^{123}I loss by binding was 1.8 ± 0.5% averaged over all the samples, and was higher with the earlier samples. When the MIBG was reduced to 200 μg per sample the labelling reaction progressed more slowly and some 14% of ^{123}I was bound. There are limitations in the accuracy of these results. One source of error is that significant times were required to heat and cool the reaction mixtures. For example, 48 sec heating was required to attain 90°C in the reaction mixture.

In the foregoing labelling results it was necessary to obtain each datum from a separate experiment (i.e. for each time three or more experiments were performed). The reason is that labelling of MIBG was arrested when a gas-tight syringe was utilized to extract aliquots at 100°C. We do not have a good explanation for this phenomenon.

The temperature dependence of MIBG labelling was measured in thin-wall glass vials at 20°C through 110°C using 2 mg MIBG and 5 mCi ^{123}I per 5 min reaction and assuming first order kinetics (unpublished data). An activation analysis gave $\Delta G^{\ddagger}/RT = 20.8$ at 100°C; the corresponding reaction coefficient (Q_{10}) is 1.7 at 100°C. A surprising result (Fig. 3) was that significant labelling took place at 20°C, although roughly 5 hours were required for the radiochemical purity to reach 95%. At 5 hours 13% of the ^{123}I was bound to apparatus.

We extended the labelling results to higher ^{123}I activities and larger solution volumes by adding sufficient extra H_2SO_4 to neutralize the additional hydroxide ions which accompanied the ^{123}I. Thus in one experiment 93 mCi ^{123}I (270 µL) was incorporated into MIBG and 99.0% radiochemical purity was obtained while the binding loss was 1.0%. In a second series dilute ^{123}I sources were prepared by suspending 5 mCi portions of ^{123}I in 1 mL 0.1 N NaOH. The alkaline solutions were acidified (with 60 µL 1M H_2SO_4), flushed, and injected directly into dry kits. Following 30 min at 100°C the radiochemical purity was 99.1 ± 0.6% (n=5) [^{123}I] MIBG while the binding loss was 4.2 ± 1.3% (n=5).

DISCUSSION

In the past Cu(I) has proven to be effective in mediating the incorporation of ^{123}I into aryl iodide radiopharmaceuticals. A current problem is to gain control of the Cu(I) reaction conditions so that excellent products may be obtained routinely. The present work with [^{123}I] MIBG initially gave puzzling results although the conditions were thought reasonable. The radiochemical purity was high (~95%[^{123}I] MIBG) while the radiochemical yield was significantly lower. Coloured solutions and precipitates were observed using these same reaction conditions. A partial explanation of the phenomena was developed.

Subsequently a procedure was found which speedily rendered high purity [^{123}I] MIBG, while artifacts were avoided.

A rationalization of the problem is that sufficent ascorbic acid and Sn(II) (in the form of $SnSO_4$) are required for maximal rate of labelling of MIBG with ^{123}I. Ascorbic acid and Sn(II) are readily altered, and then significant ^{123}I is diverted to [^{123}I]CuI precipitate as an alternative to [^{123}I] MIBG. Water, O_2, and hydroxide ion react with ascorbic acid[17] and Sn(II) leading to short effective lifetimes. Therefore the reaction ingredients were kept dry and anaerobic until the time of use, and the reaction was carried out in dilute H_2SO_4. It has been shown that Sn(II) can react in water to give a mixture of precipitable basic ions[18] (i.e. $Sn(OH)^{+1}$, $Sn_2(OH)_2{}^{+2}$, $Sn_3(OH)_3{}^{+3}$ etc.) and hydrous oxide[19] [$(Sn\ O)_5(H_2O)_3$] even when Sn(IV) is not generated. The formation of precipitable basic salts is greatly reduced[18] at pH 1 to 2. The hydrous oxide precipitates above pH 4. Mertens et al. specified[12] <10% ^{123}I by volume. This approach would limit the alkalinity, but would also limit the total ^{123}I activity.

The gross physical appearance of the final preparation proved to be a good diagnostic indicator of radiochemical quality. Preparations made as described in Materials and Methods were colourless, showed a slight turbidity attributed to tin compounds, and later proved to have ~99% radiochemical purity. Improper technique led to a variety of artifacts and lower radiochemical yields in all cases. Yellow solutions resulted from products of ascorbic acid in the absence of $SnSO_4$ or with excess O_2. Red solutions were attributed to Cu_2O derived from excess $CuSO_4$ or high pH. There is analogy to Fehling's test. White precipitates were derived from $SnSO_4$ by reaction with O_2 or hydroxide ions. Artifacts in appearance proved to be correlated to radiochemical problems, namely "bound" [^{123}I]CuI and [^{123}I] MIBG radiochemical purity less than 99%.

Although the metal salts were not removed from the pharmaceutical product, the [^{123}I] MIBG is thought to be acceptable from a toxicological standpoint. The quantity of tin is within the range (0.5 to 2.0 mg

per vial) utilized in commercial ^{99m}Tc radiopharmaceutical kits[20]. The quantity of copper is much less than the total copper in human blood (1.1 μg/mL). Up to 20 mg copper (as cupralene) has been injected intravenously into a human without ill effect[21]. The [^{123}I] MIBG product has given uniformly excellent images of pheochromocytoma and neuroblastoma in ten human patients.

It is noteworthy that the labelling has also been applied to IMP successfully. We are currently investigating the potential for ^{122}I ($T_{1/2}$ = 3.6 min) labelling utilizing ^{122}Xe from the ^{127}I(p,6n)^{122}Xe (84 MeV) reaction at TRIUMF.

In summary, we have described an accessible kit formulation and procedure for labelling [^{123}I]MIBG in the clinic. The reaction is rapid, the yield quantitative, and the procedure is viable with currently available ^{123}I concentrations.

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