Radioactive Iodine Exchange Reaction of HIPDM: Kinetics and Mechanism

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In conjunction with single photon emission computed tomography (SPECT), iodine-123 (^{123}I)-labeled N,N,N′-trimethyl-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propanediamine (HIPDM) has been used clinically as a regional cerebral perfusion imaging agent. The [^{123}I]HIPDM can be prepared by a simple aqueous exchange reaction in a kit form. We synthesized unlabeled HIPDM by condensation of 2-hydroxy-3-methyl-5-iodobezaldehyde and N,N,N′-trimethyl-1,3-propanediamine, followed by a sodium borohydride reduction reaction. The kinetics of the radioactive iodine exchange reaction for the preparation of [^{123}I]HIPDM is controlled by the pH, the temperature, and the presence of reductant (sodium bisulfite), and oxidant (sodium iodate). The reaction is a second order iodine–iodine exchange with an activation energy of 30.6 kcal/mole. The mechanism of this reaction probably involves the formation of an active I^+ or iodine free radical, which is sensitive to the presence of a reductant, such as sodium bisulfite.

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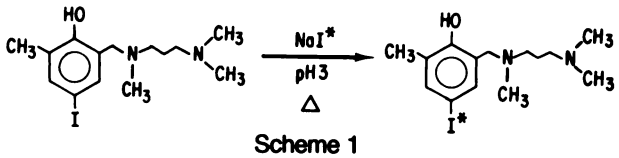
The lipid-soluble diamine N,N,N′-trimethyl-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propanediamine (HIPDM) crosses the blood-brain barrier with high first pass extraction ratio and displays a long brain retention time (*1*). In conjunction with single photon emission computed tomography (SPECT), [^{123}I]HIPDM has been used clinically for studying regional cerebral perfusion (*2–5*) and is currently in the second phase of clinical study.

The major advantage of [^{123}I]HIPDM, as compared with the other iodinated brain imaging agent—a monoamine, [^{123}I]IMP (N-isopropyl-4-iodoamphetamine) (*6–10*)—is the ease of labeling with [^{123}I]sodium iodide by a simple aqueous exchange reaction in a kit form. The hydroxy group on the benzene ring of HIPDM activates the ring thereby rendering the iodine atom more readily exchangeable (Scheme 1). In spite of the simplicity of the exchange reaction, low labeling yields have sometimes been encountered when using supposedly pure Na^{123}I. Contamination of the NaI with metal ions from the tellurium-127 (^{127}Te) target during the production of ^{123}I by a (p,5n) reaction or from rinsing the xenon trap with aqueous sodium hydroxide during the collection of Na^{123}I may affect the exchange reaction.



Scheme 1

In this report, the chemical synthesis of cold HIPDM is illustrated. The effect of the low level metal ions, temperature, and pH were evaluated. The action of the reducing agent, sodium bisulfite ($NaHSO_3$), was also studied, as this reagent is sometimes used in the [^{123}I] sodium iodide solution as a preservative. The activation energy was also determined. The information presented in this paper may be useful in understanding the basic kinetics of the iodine–iodine exchange reaction, and may also be valuable for formulating convenient kits for ^{123}I-labeled radiopharmaceuticals.

METHODS

The cold HIPDM was prepared by a method similar to that reported earlier for other iodinated phenolic diamine derivatives (*11*) (Scheme 2).

Synthesis of 5-Iodo-3-Methyl-Salicylaldehyde (II)

A solution of the 3-methyl-salicylaldehyde, I, (15 g, 110 mmol), prepared as described previously (*11*), was added

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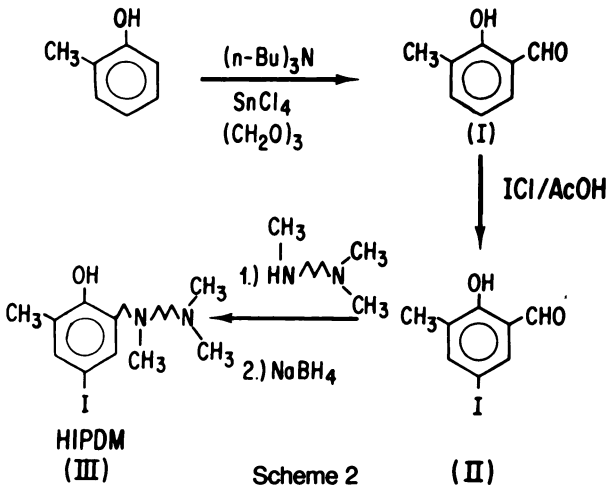

Scheme 2

dropwise to a solution of ICl (24.3 g, 150 mmol) in 100 ml of glacial acetic acid at 60°C. After the addition was completed, the dark mixture was heated for 2 hr at 75–80°C, then stirred at 55–60°C for 18 hr, after which most of the solvents were evaporated. The residue was diluted with cold water (50 ml) and filtered. The brown solid was sublimated at 55°C (0.1–0.2 torr) to give 15 g (57 mmol) of pure product (yield 52%) (*II*).

Synthesis of N,N,N′-Trimethyl-N′-(2-Hydroxy-3-Methyl-5-Iodobenzyl)-1,3-Propanediamine (III)

A solution of the iodinated aldehyde, II, (8.13 g, 31 mmol) and N,N,N′-trimethylpropane-1,3-diamine (4.1 g, 35 mmol) in 100 ml of benzene was refluxed for 30 min. The solvent was evaporated under reduced pressure to give a yellow oil. The oil was dissolved in ethanol (75 ml) and $NaBH_4$ (1.0 g, 26 mmol) was added by a spatula in small portions over 20 min. The reaction mixture was stirred at room temperature for 18 hr. The resulting clear solution was concentrated under reduced pressure, and a solution of saturated sodium bicarbonate (100 ml) and carbon tetrachloride (150 ml) was added. The carbon tetrachloride layer was separated and the aqueous layer was re-extracted with carbon tetrachloride (2 × 50 ml). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a clear oil. The oil was redissolved in 60 ml of absolute ethanol and the product was converted to the dihydrochloride salt by passing dry HCl gas through the solution at 0°C. The solution was treated with a small amount of hexane until it turned cloudy. After cooling to 4°C for 18 hr, the white crystals were filtered, dried, and recrystallized from ethanol–hexane to give 7.85 g (18 mmol) of pure dihydrochloride salt (yield 58%). The following data were recorded: ^{1}H-NMRδ (DMSO-d_6) 2.23 (S,3H,CH_3); 2.71 (S,3H,CH_3) 2.78 (S,6H,2CH_3); 3.23 (M,6H); 4.36 (S,2H,CH_2) 7.51 (D,1H,J = 2 Hz); 7.78 (D,1H,J = 2 Hz). The UV was measured as (in 0.9% NaCl): λ max 289 nm (E = 1.9 × 10^6 M^{-1}), min 266 nm. The MS (CI) was: 364 (15.6, M^{+1}); 363 (100); 362 (50.9) 247 (52.2); 87 (150). $C_{14}H_{23}H_2IO \cdot 2HCl$ elemental analysis showed: theory C:38.64, H:5.79, N:6.44; found C:38.76, H:5.88, N:6.39.

General Experimental Procedure for the Iodine–Iodine Exchange Reaction

To a solution of HIPDM (2 ml, 0.25 mg/ml) at a specific pH (0.01*M* phosphate buffer), one drop of radioactive iodide (either ^{125}I or ^{123}I, normally without iodide carrier, in 0.1 N NaOH) was added. The solution was incubated in an oil bath maintained at a specific temperature. Samples were removed at different intervals of time and assayed by thin layer chromatography (TLC) using Merck TLC plates 60F-254 and a freshly made solvent mixture of chloroform:ethanol: concentrated ammonia (8:1.8:0.2 v/v). After developing, the TLC plates were cut into 0.5 cm sections and counted in a gamma counter; R_f values for free iodide and labeled HIPDM were 0.01 and 0.5–0.8, respectively. Iodine-125 and ^{123}I gave the same results for the exchange reaction.

Most of the kinetic studies reported in this paper were carried out at a lower concentration (0.25 mg of HIPDM/ml) than that of a clinical kit (2 mg/ml). At the lower concentration, the reaction rate is slower, so the reaction kinetics can be measured more adequately.

Effects of Temperature

The reaction solution was buffered at pH 3.0 and immersed in a constant temperature bath. The reaction solution was sampled at various time points and analyzed using the TLC method described above. The exchange reaction was studied at 40, 50, 60, 70, 80, 90, and 100°C.

Effects of Sodium Iodide Concentration

The exchange reaction was carried out in the presence of increasing amounts of sodium iodide to evaluate if it is a second order reaction. The sodium iodide concentration varied from 0 μg/ml (carrier-free) to 66 μg/ml.

Effects of pH

The exchange reaction was studied at various pHs ranging between 1 and 14. At lower pHs (pH 1 and 2) a citrate buffer was employed, whereas at higher pHs (pH 13 and 14) a solution of sodium hydroxide was used. Phosphate buffers were used for pHs between 3 and 12. Reaction temperature was held at 100°C and samples were analyzed at 30 min.

Effects of Trace Metal Ions

The reaction was carried out in the presence of different metal ions at concentrations indicated in Table 1. The reaction temperature, time, and pH were 100°C, 30 min, and 3.0, respectively.

Effects of Sodium Bisulfite or Potassium Iodate

The exchange reaction was studied under the following condition: HIPDM (1 ml, 2 mg/ml in 0.01*M* phosphate buffer), pH 3, heated at 100°C for 30 min. Each reaction was carried out in the presence of reducing agent or oxidizing agent at a concentration specified in Table 2.

RESULTS AND DISCUSSION

Effects of Temperature

There is a significant effect of temperature on the exchange reaction rate (Fig. 1). When the reaction temperature is lower than 90°C, the reaction did not reach 95% in < 1 hr of heating. At 100°C, the reaction reached 95% in < 30 min. The fraction of exchange (F)

TABLE 1
Effects of Sodium Iodide Concentration

	HIPDM*			NaI†				
	Volume (μl)	Weight (μg)	Final concentration (μM)	Volume (μl)	Weight (μg)	Final concentration (μM)	Expected yield‡	Yield found
A	100	100	2.30	0	0	0	100.0	97.2
B	100	100	2.09	10	3.4	0.22	90.9	86.1
C	100	100	1.91	20	6.8	0.38	83.4	78.1
D	100	100	1.84	25	8.5	0.45	80.1	72.9
E	100	100	1.53	50	17.0	0.75	66.9	58.9
F	100	100	1.15	100	34.0	1.13	50.3	44.9
G	50	50	0.77	100	34.0	1.51	33.6	30.4

*HIPDM stock 1.0 mg/ml + 0.5 μg/ml NaI (kit).
†NaI stock 0.340 mg/ml.
‡The expected yield is ([HIPDM]/[HIPDM] + [I^-]) × 100%.

was calculated based on the following equation:

$$F = \frac{[HIPDM^*]_t - [HIPDM^*]_0}{[HIPDM^*]_\infty - [HIPDM^*]_0}, \quad (1)$$

$[HIPDM^*]_t$ = radioactive HIPDM at time t

$[HIPDM^*]_0$ = radioactive HIPDM at time 0

$[HIPDM^*]_\infty$ = radioactive HIPDM at time ∞

The $[HIPDM^*]_\infty$ was determined at 24 hr after the initiation of the exchange reaction. At time zero, the amount of radiolabeled HIPDM was close to zero. Therefore:

$$F = \frac{[HIPDM^*]_t}{[HIPDM^*]_\infty}.$$

When ln(1 − F) is plotted as a function of time, a straight line is obtained for all reaction temperatures studied (Fig. 2). This strongly suggests that the mechanism of this exchange reaction is a simple second order iodine–iodine isotope exchange reaction. The reaction rate appeared to be very dependent on the concentration of HIPDM and sodium iodide (see data presented below). At higher HIPDM concentration (2 mg/ml), a concentration used in the current kit formulation, the exchange reaction is usually completed within 5 min at 100°C.

TABLE 2
Effect of Trace Metal Ions on the HIPDM Exchange Reaction

Trace metal	Chemical formula	Quantity (mg/ml)	Labeling yield (%)
None	—	—	98.3
Fe^{+3}	$Fe(NO_3)_3 \cdot 9H_2O$	1.47	95.2
Cu^{+2}	$CuSO_4 \cdot 5H_2O$	1.31	91.5
Al^{+3}	$Al_2(SO_4)_3$	1.63	96.8
Zn^{+2}	$Zn(Ac)_2 \cdot 7H_2O$	1.51	95.6
Mg^{+2}	$MgCl_2 \cdot 6H_2O$	1.55	96.7
Ni^{+2}	$Ni(NO_3)_2 \cdot 6H_2O$	1.48	97.6

Effects of Sodium Iodide Concentration

The concentration of sodium iodide clearly showed a significant effect on the labeling yield. It is apparent from the data presented in Table 1 that the reaction is a second order reaction. The expected yield is quite consistent with the calculated yield based on second-order reaction kinetics.

Effects of pH

When the reaction pH is below 6, the HIPDM exchange reaction proceeds smoothly, as there is no sig-

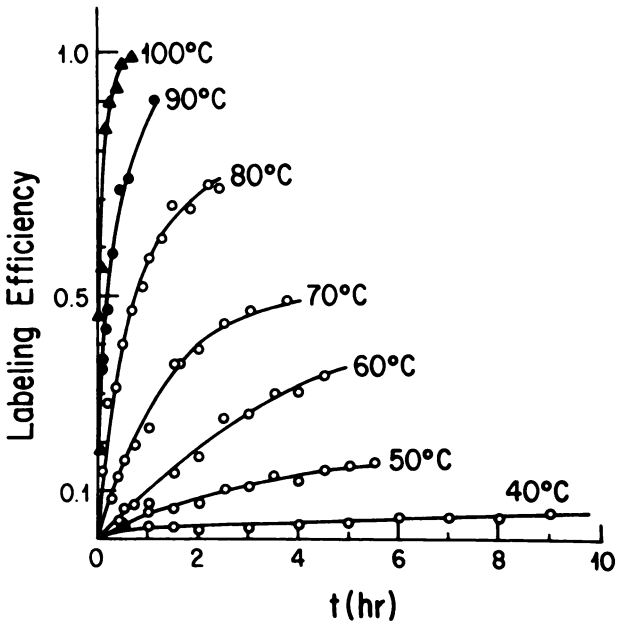

FIGURE 1
Effects of temperature on labeling efficiency at various time points.

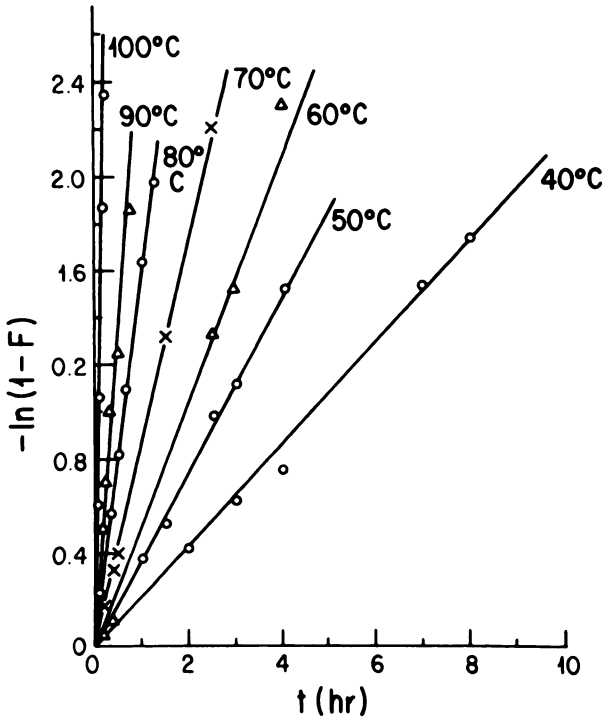

FIGURE 2
Effects of temperature on labeling efficiency. The same data points from Figure 1 but presented as a plot of ln (1-F) vs. time (F:fraction exchanged). The straight lines for every temperature studied indicate that the reaction is a simple iodine-iodine exchange reaction.

nificant difference in the labeling yield. When the pH is above 6, however, the exchange reaction shows a sharp drop in the labeling yield (Fig. 3). This is probably due to the formation of I^- anion at pHs higher than 6, which prevents the exchange reaction. The optimum pH for this exchange reaction is around pH 3. In this report, except as noted, all of the HIPDM exchange reactions are carried out in pH 3.0 buffered solutions. The huge difference in labeling efficiency between pH 7 and pH 8 may be related to the deprotonation of amines. A similar type of curve was reported for labeling ^{123}I iodoantipyrine, but the dramatic shift in labeling yield occurred at lower pH (~pH 3–4) (*12*).

Effects of Trace Metal Ions

Trace metal ions such as Fe^{+3}, Cu^{+2}, Al^{+3}, Zn^{+2}, Mg^{+2}, and Ni^{+2}, may form a complex with HIPDM and affect the exchange reaction. When the reaction is carried out in the presence of trace metal ion, there are no significant changes. The only exception is Cu^{+2}, which lowers the yield to 91.5% (Table 2). It is not clear why only Cu^{+2} has the ability to inhibit the reaction while all of the other trace metals do not.

Effects of Reductant and Oxidant

The effect of a reductant, sodium bisulfite ($NaHSO_3$), a common preservative for no carrier added radioactive sodium iodide, on the exchange reaction was evaluated. The results in Table 2 indicate that at a concentration of 20 μg/ml, sodium bisulfite effectively stops the exchange reaction. The inhibitive effect is reversed by adding an oxidant such as potassium iodate (KIO_3), at a concentration of 20 μg/ml (Table 3). The above results suggest that the reaction mechanism may involve the formation of an active I^+ or iodine free radical. In the presence of a reductant, the I^+ or iodine free radical can no longer exist and inhibition of the exchange reaction occurs. However, when the reducing effect is neutralized by an oxidant, then the exchange reaction does take place again in high yield (see Table 3). The presence of I^+ as an active intermediate is an important factor to consider when designing a kit formulation for routine

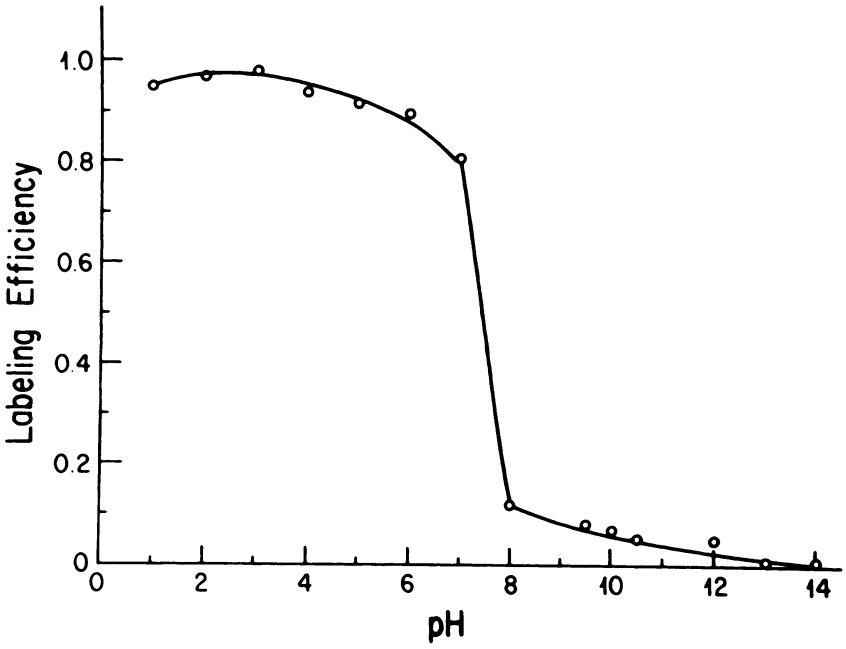

FIGURE 3
Effects of pH on the labeling yield. The labeling yield declines significantly when the reaction pH is above 6.

TABLE 3
Effect of Reductant and Oxidant* on the HIPDM Exchange Reaction

$NaHSO_3$ (μg)	Labeling yield (%)†	Reductant $NaHSO_3$ (μg)	Oxidant KIO_3 (μg)	Labeling yield (%)†
0	98.1	0	20	97.9
1	95.5	20	0	0.6
5	97.5	20	20	96.5
10	97.4	20	40	96.5
20	0.5	20	100	96.6
30	0.3			
40	0.2			
50	0.1			
200	0.4			
500	0.6			
1000	0.0			

* Sodium bisulfite was the oxidant used.
† Average of two experiments.

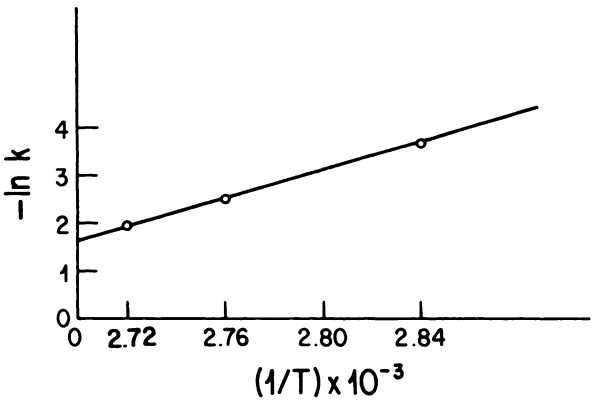

FIGURE 5
A plot of reaction rates vs. 1/T (T:absolute temperature, K°). The activation energy Ea is determined by fitting the data with the Arrhenius equation. Slope = 1.541×10^4; ΔE_a = 30.6 kcal/mole.

nuclear medicine use. Proper precaution should be taken to avoid the contamination of reductants, which will inhibit the exchange reaction.

Activation Energy for the Exchange Reaction

The carrier-added exchange reactions, using 0.1 mg of potassium iodide and 0.5 mg/ml of HIPDM at pH 3, were studied at three different temperatures, 79°C, 89°C, and 94°C. The reaction rate at each temperature was measured and the second-order rate constants (k) were determined (Fig. 4). By measuring the slope for a plot of ln k versus 1/T and fitting the Arrhenius equation:

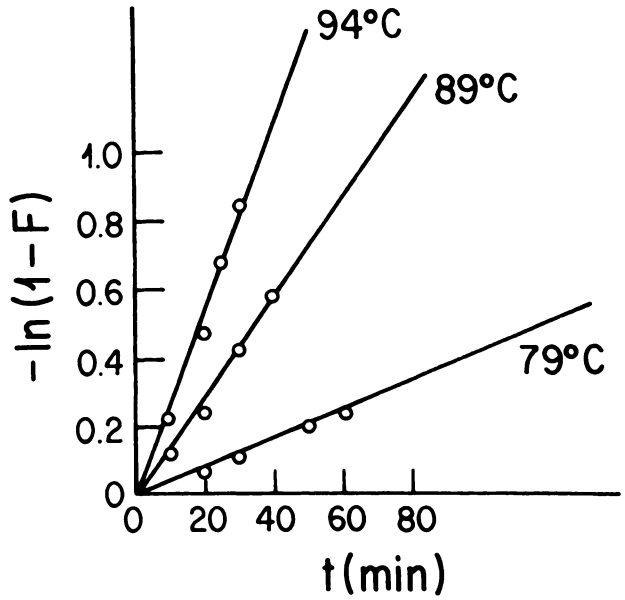

FIGURE 4
Measurement of reaction rates (the slope of each line) at different temperatures under a carrier-added condition.

$$\ln k = \frac{Ea}{RT} + \ln A, \qquad (2)$$

where R is the gas constant, T is the absolute temperature, the activation energy Ea can be calculated. As a result, Ea was determined to be 30.6 kcal/mole (Fig. 5).

The kinetics of the iodine–iodine exchange reaction of HIPDM have been evaluated. Based on the effects of pH and temperature and the presence of reductants and oxidants, the reaction probably is a second order iodine–iodine exchange reaction with an activation energy of 30.6 kcal/mole. The mechanism of this reaction may involve the formation of an active I^+ or iodine free radical. The information may be of use in designating new radioiodine labeled radiopharmaceuticals in a kit form for regular nuclear medicine clinics.

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