

CONF-8509264--1

BNL 37642

Invited Presentation
Accepted For Publication, International Journal Of Applied Radiation & Isotope

Received by OSTI
MAR 18 1986

123; RESEARCH AND PRODUCTION AT BROOKHAVEN

L. F. Mausner, S. C. Srivastava, S. Mirzadeh, G. E. Meinken
and T. Prach

Medical Department, Brookhaven National Laboratory
Upton, NY 11973

BNL--37642

DE86 007816

Presented in part at the International Symposium on Radiohalogens,
Banff, Alberta, Canada, 10-11 September 1985.

Research supported under the U.S. Department of Energy Contract
No. DE-AC02-76CH00016.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

774

ABSTRACT

The procedures for preparing high purity ^{123}I at the BLIP using the $^{127}\text{I}(p,5n)^{123}\text{Xe}$ reaction on an NaI target are described. The activity is supplied in a glass ampoule with anhydrous ^{123}I deposited on the interior walls, allowing maximum flexibility in subsequent iodinations. Preliminary experience with a continuous flow target is also described. The results of a series of measurements of specific activity by neutron activation, x-ray fluorescence, uv absorption, and wet chemistry generally showed no detectable carrier. HPLC methods to analyze the chemical form of radioiodine and to characterize various iodinated radiopharmaceuticals have been developed. These methods provide higher sensitivity, speed and resolution than commonly used techniques.

INTRODUCTION

This paper summarizes our research on the production of high purity ^{123}I , research on new and improved quality control and chemical characterization techniques, and studies to optimize labeling conditions. The radiopharmaceutical community has long recognized the usefulness of high purity ^{123}I in diagnostic nuclear medicine⁽¹⁾. Its advantages involve high chemical reactivity and favorable decay characteristics including no β emission, short half-life, and emission of a 159 KeV gamma ray well suited to available imaging instrumentation. The availability of 200 MeV protons at the Brookhaven Linac Isotope Producer (BLIP)⁽²⁾, has for many years facilitated the production of this neutron deficient nuclide using the $^{127}\text{I}(p,5n)^{123}\text{Xe} \rightarrow ^{123}\text{I}$ reaction⁽³⁾. The ^{123}I is prepared in a unique anhydrous form. Measurements of yield and radiopurity are reported for a batch mode of operation. Because of anticipated higher yield we have begun to investigate the construction and operation of a continuous-flow processing system.

The specific activity and chemical form of the ^{123}I often have a significant effect on the success of many iodination reactions. We describe a new method for the isolation and characterization of many common inorganic as well as organic forms of iodine using the highly sensitive HPLC technique⁽⁴⁾. We also report on the application of neutron activation, x-ray

fluorescence, uv absorption and wet chemical procedures to determine the specific activity of ^{123}I . Finally, results on the effect of iodine form on iodination procedures and the optimization of labeling conditions for some routinely used radiopharmaceuticals are included.

EXPERIMENTAL

Procedure for ^{123}I Production

The target is a disk of NaI 0.89 cm thick and 7.0 cm in diameter, weighing approximately 125 g. It is made by drying NaI powder under vacuum and heat for 16 h and then pressing this powder in a die at 70 tons pressure. This disk is encapsulated in a stainless steel disk with an outer diameter of 10.1 cm and 0.64 cm wall with 0.025 cm inconel windows electron beam welded under vacuum. The salt pellet is centered in the disk with a folded stainless steel spring. An inconel fitting with a thin diaphragm covering its base is welded over a hole in the rim of the capsule. This allows connection to the gas processing equipment. All finished targets are visually inspected and leak checked.

The irradiations are performed at the BLIP which utilizes the excess beam capability of a linac that injects 200 MeV protons into the 30 GeV Alternating Gradient Synchrotron. The linac provides BLIP with an integrated beam current of approximately 50 μA of protons with 200 MeV energy, which is high enough to allow simultaneous irradiation of multiple targets in a thick stack array. There are seven independently moveable, stainless steel

target holders, each of which can hold 1 to 3 salt targets (depending on their thickness) plus several thin foil targets. The NaI target is typically positioned in the stack to receive 68.6 MeV protons and is typically irradiated for 3 hours.

After irradiation, the target is removed from BLIP and transported in a shielded cask to a remote processing facility. There it is attached to a stainless steel gas train shown in Figure 1 by means of the gas fitting on the target rim. A leak-tight fit is obtained by compressing an annular "C" seal made of inconel between the target fitting and the receptacle on the gas train. All valves are remotely operated pneumatic valves. The inconel diaphragm at the base of the fitting is then ruptured with a remotely controlled hollow needle assembly and the capsule is heated to 750° to melt the sodium iodide. Xenon diffusing out of the molten salt is swept out of the target by a helium stream flowing at ~15 ml/min and is carried through a series of traps to purify the xenon. First is a steel U-tube at -78° to remove condensables such as NaI vapor, I₂, and water vapor. Next, the gas passes through a trap containing silver filings maintained at 400° and then through a silver loaded silica gel trap at 100°, both to react with any traces of iodine contamination. Then, xenons are condensed in a stainless steel spiral at -196°. This trap is backed up with a silica gel trap at -196° to prevent traces of radioxenons from following the helium into the ventilation system. The target sweep continues for 30 min and removes in excess of 90% of the xenon in this time. Thirty

minutes has been chosen as a compromise between removing the last traces of xenon from the target and decay loss. The activity of both the xenon trap and the backup trap is monitored during xenon collection by Geiger counters.

After collection, the ^{123}Xe in the spiral trap is cryogenically transferred to an evacuated glass ampoule⁽³⁾. After warm-up, the gas in this ampoule is equilibrated with up to 9 other ampoules and refrozen at -196° . The volume of the ampoules then determines the amount of xenon in each and thus the eventual ^{123}I activity. These ampoules have been arbitrarily fixed at two sizes to yield for use at receipt 10 and 20 mCi of ^{123}I . The xenon is allowed to decay in the ampoules, typically for 5 h, and then warmed to room temperature and pumped off. Finally, the valve stem connecting the ampoule to the gas rack is flame sealed and the ^{123}I activity is assayed in a dose calibrator with a copper liner after any ^{122}I has decayed. The ^{123}I can be removed from the ampoules by forcing a syringe needle through the septum, breaking the internal break seal, and injecting as little as 0.2 ml of a suitable solvent. Enhanced recovery is usually attained with a second rinse.

Investigations have begun to construct a processing system wherein helium gas flowing over a molten sodium iodide target continuously sweeps out ^{123}Xe produced during bombardment. A test target has been developed, shown in Figure 2. The target

consists of an inner stainless steel capsule and an outer aluminum can. The inner vessel holds the sodium iodide target salt, and contains two heating elements, a thermocouple and a line for helium to flow across the salt surface. The outer can is primarily a secondary containment vessel to prevent irradiated salt from getting out if the inner vessel leaks.

The target is connected to the Chemistry Linac Irradiation Facility (CLIF)⁽⁵⁾ operating floor by a 30-foot hose and cable bundle. There are two helium inlet hoses, one helium and radioactive xenon outlet hose, electrical lines for the heating elements and thermocouple and an outer containment hose. The bottom 6-12 inches of the helium lines are stainless steel, changing to PVC for the rest of their length. Similarly, the outer hose is 18 inches of stainless steel mesh connected to PVC.

During irradiation, helium from a tank flows past the target salt and sweeps out xenon radioactivity produced by $^{127}\text{I}(p,xn)$ reactions. The gas then flows through a silver-silica gel trap to scrub possible iodine vapor, through a coil placed close to a Ge(Li) detector coupled to a multichannel analyzer (MCA), and into three liquid nitrogen-cooled traps which remove all the xenon. Then the helium flows through a hold up volume filled with activated charcoal and is finally vented. A radiation detector

monitors this volume. The secondary flow runs from a tank through the space between the inner and outer containment capsules. This helium flows through a liquid nitrogen-cooled silica gel trap and then into a hold up volume not shown. Radioactivity sensed at this silica gel trap would imply a leak of the inner target.

Quality Control and Characterization of ^{123}I

Radionuclidic Purity. Radiopurity is determined with an intrinsic Ge detector calibrated against known solution sources (^{154}Eu , ^{155}Eu , ^{125}Sb) obtained from the National Bureau of Standards. The counting sample is a 1-ml aliquot taken from a dilution of an ampoule wash solution. Measurements are performed approximately 24 h after the end of bombardment and many days later after complete decay of ^{123}I .

Specific Activity. Several different methods were investigated to determine the ^{123}I specific activity. These were neutron activation, wavelength dispersive x-ray fluorescence, uv absorption at 225 nm, and a colorimetric method that makes use of the catalytic effect of iodine on the reaction of ceric sulfate with arsenious acid.

For neutron activation, a quartz ampoule (~1 mm I.D., 6 mm O.D., and ~4 cm in height) containing an aliquot of ^{123}I (produced through the decay of ^{123}Xe directly in the ampoule) was allowed to decay for several days. This ampoule together with a blank and two standard ampoules each containing 1.9 μg of iodine (as NaI) were then irradiated at the reflector location of the Brookhaven

High Flux Beam Reactor (HFBR) with a neutron flux of 1.5×10^{14} s⁻¹cm⁻² for a duration of 5.0 min. After 30 min of cooling, gamma-ray spectra of the ampoules were taken at fixed geometry with a Ge detector (~50 cm³, FWHM ~1.8 at 1333 KeV) coupled to a 4096 channel MCA.

In another run, the activity of an ampoule (1 of 5) was rinsed out in 0.6 ml acetone. To this solution, 1 ml of 0.1 N NaOH was added and the acetone allowed to evaporate. The solution was diluted to 25 ml with water and passed through ion exchange paper (SB-2). The iodide content of this sample was measured in a wavelength dispersive x-ray fluorescence spectrometer by comparison with a standard curve.

The iodide-catalyzed reaction of ceric ion with arsenious acid⁽⁶⁾ was also utilized for determining the carrier content of ¹²³I samples. A calibration curve was generated using appropriate standards containing known amounts of iodide in the range of 0-10 ng/ml. The system obeyed Beer's law within this concentration range and provided a linear plot of absorbance vs. concentration at 420 nm.

Measurement of absorbance at 225 nm was another method that was successfully used to determine the iodine content in ¹²³I samples from various runs. This method also allowed the monitoring of HPLC-fractionated samples. Since many species of iodine do not absorb at 225 nm, the measurement yielded values mainly for those fractions of ¹²³I which were present as iodide, iodate, or periodate. In most runs, the iodide content accounted for >95% of the activity in the samples. Due to the sensitivity

of the technique, and the fact that many impurities may absorb at 225 nm, the reagents and blanks were prepared under extremely careful conditions. The standard curve showed a linear relationship between 0.01 and 10 μg iodide.

HPLC Methods for Radiochemical Purity Determination.

HPLC methods were developed in order to determine the chemical nature of iodine in ^{123}I samples. Routinely used TLC and paper chromatographic procedures⁽⁷⁾ were also utilized to compare the relative sensitivity, speed, and resolving capacity of the HPLC method. Commercial ^{131}I and ^{125}I samples were also subjected to these analyses to serve as references for comparison studies.

A number of reverse-phase columns (C_2 , C_8 , C_{18}) and solvent mixtures were evaluated for optimal separation efficiency. A Waters' Associates liquid chromatograph with a dual solvent programmer was used. An on-line absorbance detector set at 225 nm was utilized to monitor the carrier iodine content of the various separated fractions. A radioactivity flow monitor (NaI detector) coupled to a ratemeter was used for measuring the radioactivity of fractions during elution. Additionally, fractions were collected in sample tubes and subsequently counted in a well-type gamma counter for validation of the flow-detection results. The eluting buffers (pH 7.0) contained 0.05 M phosphate, 0.002 M tetrabutylammonium hydroxide, and varying concentrations (10-40%) of methanol or acetonitrile. The flow rate was 1-2 ml per min and all studies were carried out at 22° using 4.6 x 250 mm columns. The ^{123}I samples were also subjected to cycles of oxidation and

reduction using hypochlorite, hydrogen peroxide, chloramine T, thiosulfate, metabisulfite, H_2S , and SO_2 . Separation of iodate, iodide, and periodate was studied using freshly prepared synthetic mixtures from high-purity reagent-grade chemicals. Elution factors ($k' = \text{elution volume} - \text{column volume} / \text{column volume}$) were established for IO_3^- , I^- , IO_4^- , I^+ , CH_3I , and CHI_3 using appropriately characterized standards. No deviations in k' values were observed when various size samples (volume or concentration) were used for HPLC injection.

Radioiodinations Using ^{123}I .

BLIP-produced ^{123}I from various runs was used to prepare three commonly used radiopharmaceuticals: N-isopropyl-p-iodoamphetamine (IAMP), N,N,N'-trimethyl-N'-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propane-diamine (HIPDM), and deoxyuridine (IUDR). Commercial ^{131}I and ^{125}I solutions were used in parallel experiments for the sake of comparison and validation of the data. IAMP was iodinated using the $CuSO_4$ catalyzed exchange reaction in refluxing glacial acetic acid⁽⁸⁾. Essentially carrier-free IAMP was prepared by iodinating bromo-amphetamine (BRAMP) followed by HPLC separation of IAMP and BRAMP on a C_{18} reverse-phase column using pH 7, 0.05 M phosphate in 40% \rightarrow 60% methanol. Both radioactive detection and uv detection at 265 nm were utilized. HIPDM was iodinated using the kit method of Kung and coworkers⁽⁹⁾. A satisfactory HPLC purification was achieved using a C_{18} reverse-phase column and a solvent mixture containing 0.05 M phosphate in 30% acetonitrile, pH 5. IUDR was iodinated using an exchange reaction in 1 M HNO_3

under heating. HPLC separation of the product was accomplished on a C₁₈ reverse-phase column using pH 5, 0.05 M phosphate buffer in 10% methanol. Concomitant TLC analyses were performed on iodinated IAMP and HIPDM using the following eluting mixtures: chloroform, glacial acetic acid, methanol (15:1:85 v/v) (IAMP); and chloroform, ethanol, ammonia (8:2:0.1 v/v) (HIPDM).

Biodistribution Studies.

Tissue distribution studies in mice of radiolabeled IAMP and HIPDM were carried out with both ¹³¹I (commercial) and ¹²³I (BLIP-produced)-labeled materials. Normal BNL mice (n=4 for each experiment) were injected with 0.2 ml of the labeled compounds containing approximately 2 μ Ci ¹³¹I or 15 μ Ci ¹²³I, and sacrificed at 5, 60, and 120 min after injection. Tissue samples (or whole organs) were collected, weighed, and counted in a well type gamma counter. The data were represented variously as per cent injected dose per organ or per gram tissue, and excretion was calculated by difference using the amount injected and the total recovered activity in all organs, tissue samples, and the carcass. All data were tested for statistical significance.

RESULTS AND DISCUSSION

After penetrating through several windows, water gaps, and upstream targets, the proton energy incident on the NaI salt is degraded to 68.6 MeV and dissipates a further 21.0 MeV in this target. This energy range encompasses the peak of the $^{127}\text{I}(p,5n)^{123}\text{Xe}$ excitation function⁽¹⁰⁻¹³⁾ and represents a compromise between maximizing ^{123}Xe production and minimizing the unwanted $^{125},^{121}\text{Xe}$. Energy loss calculations have been performed by logarithmic interpolation from data of Janni⁽¹⁴⁾, and Williamson et al ⁽¹⁵⁾, with Bragg's additivity rule assumed for compounds.

The thick target yield of ^{123}I has been calculated to be 4.3 mCi/ μAh for conditions approximating our batch production, based on the cross-sections of Syme et al ⁽¹⁰⁾. These conditions are: 3 h irradiation, 2 h target transport and gas transfer time, and 5 h ^{123}I growth period. Although actual experimental parameters rarely match these conditions exactly, our measured yield averages 3.3 mCi/ μAh . Some of this loss is due to residual xenon not leaving the target, and the possibility that some xenon may not trap in the stainless steel collection coil due to the relatively high vapor pressure of xenon at liquid nitrogen temperature. However, large amounts of xenon getting past this trap would be detected at the cold silica gel trap. Some ^{123}I in a volatile form might be lost when xenon is pumped from the glass ampoule. However, passing this xenon through an iodine trap has not proved this speculation. Although the proton energy is not optimum for

the $^{127}\text{I}(p,6n)^{122}\text{Xe}$ reaction, useful quantities of ^{122}Xe ($t_{1/2} = 20.1$ h) are also produced in this short bombardment and have been utilized for generating ^{122}I , a 3.6 m half-life positron emitter. At the end of the ^{123}I growth period there is typically still ~80 nCi of ^{122}Xe available to generate ^{122}I (16).

The radiopurity is controlled in several ways. First, the incident energy and target thickness are chosen based on excitation functions for $^{121,122,123,125}\text{Xe}$. Second, the duration of bombardment and the xenon decay period are appropriately chosen. Lengthening these periods increases the production of ^{123}I but also of the impurity ^{125}I . A final practical consideration is that the irradiation, processing, analysis, and shipping must all be accomplished on the same day. With 2 to 3 h irradiations and 4 to 5 h growth periods, the average contamination is 0.5% ^{125}I and 0.002% ^{121}Te at calibration time (noon day of receipt), which is typically 21 h after xenon-iodine separation. There is no ^{124}I .

The glass ampoule offers the user several advantages. The iodine is adsorbed on the walls under anhydrous conditions and can be rinsed off with a wide variety of solvents compatible with subsequent iodination steps. Solvents such as dilute NaOH, methanol, Na_3PO_4 , NaHCO_3 , and even water have been successfully used, removing an average of 90% of the activity in <0.6 ml total volume (two 0.3 ml washes). In our experience, acetone should be avoided due to activity losses, possibly due to formation of a volatile iodine such as iodoform. Exchange labeling of

iodoamphetamine was also poor following an acetone rinse of the ampoules.

Another advantage of this dispenser is that the ampoule itself can be used as an enclosed reaction vessel, eliminating the transfer step. These benefits to the user come at some cost to the producer. The extra time involved in moving the xenon to the ampoules adds to xenon decay loss and thus reduces ^{123}I yield. All ^{123}I created before the xenon reaches these ampoules is lost. Also, xenon moves sluggishly so it is not possible to wait for full equilibration between ampoules. Thus, control of the amount of activity per ampoule is inexact. At one time, 0.5 atmosphere of H_2S was added during xenon decay to act as a reducing agent and thus to increase the ^{123}I -iodide recovery from the ampoules. More recent experience has shown that scrupulous cleaning of the ampoules makes H_2S unnecessary, thus avoiding possible iodination problems due to the remaining traces of this gas.

The theoretical specific activity of pure ^{123}I is 1.961 mCi/ng. Diluted by our measured level of ^{125}I contamination this becomes 1.957 mCi/ng (or 0.51 ng/mCi). Various techniques with different sensitivities have been investigated to measure the iodine carrier mass at nanogram levels.

Neutron activation is a sensitive and selective method for the determination of sub-microgram amounts of iodine. This is due to a) very large thermal neutron capture cross-section of ^{127}I , 6.2 barns; b) short half-life of product nuclide, ^{128}I , $t_{1/2} = 20.0$ min; and c) emission of a rather energetic gamma ray, 442.9 KeV, by ^{128}I with emission probability of 17.5%. Instrumental

neutron activation also offers an advantage over other analytical techniques in that the possibility of contaminations introduced by impurities in the chemical reagents and/or by chemical manipulation are reduced to a minimum.

After neutron exposure, no gamma-rays were observed at 442.9 keV in either an aliquot of the ^{123}I sample or the blank. However, by comparison of the magnitude of the background in the ^{123}I sample spectrum with the counting rate of the 442.9 keV peak in the spectra of the standards, an upper limit of 30 ng of iodine was estimated. By extrapolating this measurement to the whole sample, the specific activity is calculated to be <0.75 ng/mCi at the end of ^{123}I growth; essentially carrier free. Although this sensitivity can be improved, due to the time and expense of neutron activation, other techniques were tried. X-ray fluorescence on another sample showed no iodine down to 100 ng level, giving an upper limit of specific activity of 2.5 ng/mCi. Measurement of iodine mass following the uv absorption at 225 nm of the HPLC separated fractions is a convenient procedure with a 10 ng sample detection limit. Typically, 1 to 4% of the total activity was used for this determination. In 12 irradiations there was no detectable iodine, with a calculated specific activity range of 1.25-16 ng/mCi. In 3 other runs an average of 127 ng was measured, representing a dilution of radioactive atoms with stable atoms by factors ranging from 14-100. Finally, in one run the iodine mass was measured by the iodine-catalyzed reduction of ceric sulfate by arsenious acid. A value of 66 ng was obtained representing a dilution factor of approximately 60. Although the

sensitivity of this technique is very high (~1 ng detection limit), the actual procedure is time-consuming and requires extreme experimental care. Thus in approximately 80% of the runs measured there was no detectable carrier iodine.

The batch mode is a relatively labor intensive procedure and involves substantial decay losses due to the time required to transport the target to the Hot Laboratory for processing, and the transfer of ^{123}Xe to the glass ampoules. Although we have long recognized that an on-line continuous-flow method could produce much greater ^{123}I yield with less manpower, the previous physical arrangement of BLIP made this technique impractical. However, BLIP II has been designed to be more compatible with such an endeavor. A continuous-flow target of equal effective thickness to the batch target would produce a theoretical yield of 17.3 $\text{mCi}/\mu\text{Ah}$ compared to 4.3 $\text{mCi}/\mu\text{Ah}$ at present (based on the excitation function of Syme et al.). In addition, the continuous-flow target could be reusable.

The exploratory phase of this project has been performed at the CLIF, which shares the BLIP beam line but offers much simpler access. A test target has been developed, shown in Figure 2. Several preliminary runs with the continuous-flow NaI target have been completed. We were primarily concerned with the behavior of the target itself, not the collection system. The quantity of ^{123}I activity in the collection coil is a complicated function of gas flow rate, trapping efficiency, bombardment time, and decay time, as well as ^{123}Xe yield from the target. Changes in target behavior are not immediately seen in the collection coil due to

these other obscuring variables. Therefore we did not monitor for iodine radioactivity in the trap but rather concentrated on the emission of xenon radioactivity from the target. For this purpose, the most useful radionuclide was found to be ^{125m}Xe ($t_{1/2} = 57$ s), which gave a clean signature (112 keV gamma ray) in the flow coil. A study of the yield of ^{125m}Xe as a function of the duration of irradiation indicated a two-phase release mechanism at temperatures less than melting. There was an initial rapid growth of activity, corresponding to a half-time of about one min, followed by a much slower step. The slow increase of ^{125m}Xe activity continued even after thermal equilibrium was reached. This behavior could be a consequence of local melting of the NaI in the beam strike area followed by a slow diffusion of xenon through surrounding unmelted target crystal. Careful control of target temperature was accomplished with electric heaters, and the beam current was limited to 1-2 μA . Below the melting point of NaI, the increase in target temperature due to beam heating alone was $80 \pm 10^\circ$ per μA of beam current. This interval was essentially independent of initial target temperature. The yield of ^{125m}Xe increased with final target temperature, showing a sharp increase at approximately 300° . The effect of helium flow rate on yield was also investigated. A flow rate of 50 ml/min was found most convenient but this result pertains only to ^{125m}Xe yield and not necessarily to optimum conditions for ^{123}I collection.

The newly developed HPLC methods for the determination of chemical nature of iodine in ^{123}I samples proved to be highly sensitive, rapid, and reproducible. Not only better resolution

was obtained compared to other routine techniques but many new and previously unresolved species could be separated as well. Using uv detection at 225 nm, the iodine carrier content of various fractionated samples could also be measured down to 0.01 μg levels. Radioactivity detection mode in addition to offering much higher sensitivity also allowed the identification of non uv-absorbing species of iodine. Commercial ^{131}I and ^{125}I samples gave results that correlated well with TLC results and with the known carrier content of these solutions.

Typical results of HPLC experiments are summarized in Table 1. Separation of iodate, iodide, and periodate in a freshly prepared synthetic mixture (upon standing, the periodate oxidizes iodide to produce iodate) is depicted in Figure 3. Methanol was used in earlier studies but acetonitrile was later found to be more effective. Even though either of the C_2 , C_8 , or C_{18} columns could be used, C_8 columns displayed the right polarity for good and rapid separations. The elution diagram in Figure 3 was obtained for a 30 μl injected sample containing 2 μg I^- , 4 μg IO_3^- , and 10 μg IO_4^- . A quantitative correlation was obtained from measurements of absorbance after correcting for differences in molar absorptivity at 225 nm of the various species. Figure 4 shows the elution profile of an actual ^{123}I sample following oxidation with 5% sodium hypochlorite under basic conditions. The

iodide fraction (initially >95%) decreased to 12.1% and 9.3% iodate was formed. In addition, three unknown higher oxidation state species x, y, and z (25.8, 15.9, and 31.1%, respectively) were also produced. When this same sample was oxidized with hypochlorite at pH 2 (Fig 5), iodide was decreased to 2.2% and 67.7% iodate was produced. Species y was absent while species x and z accounted for 13.3% and 12.3%, respectively, of the total eluted radioactivity. In another experiment, I^+ was produced (as ICl) from a reaction of $^{123}IO_3^-$ with 0.1 N HCl for 5 min. The elution factor for I^+ was found to be 7.0 using 10% acetonitrile and other buffer components as above, and the RP8 column (Table 1). When the ^{123}I samples (containing >95% I^-) were subjected to cycles of oxidation and reduction using H_2O_2 , chloramine T, thiosulfate, metabisulfite, H_2S , SO_2 , etc., formation of a number of different species was demonstrable. In addition to the known species (IO_4^- , IO_3^- , I^+ , I^- , etc.), at least six other unknown species in varying amounts for particular reactions were documented.

The HPLC method thus allows a convenient and rapid separation of iodine in multiple chemical forms, with high resolution. Measurement of the carrier iodine content using uv detection at 225 nm, however, requires considerable care because of the preponderance of many impurities absorbing at this wavelength as

well as the fact that commonly used reagents and solvents often contain appreciable amounts of iodine as a contaminant. All solutions, blanks, and standards have thus to be prepared under extremely careful conditions.

Figure 6 shows the elution diagram of an ^{123}I sample from another manufacturer. This sample contained 84% iodide and 12% iodate. In comparison, BLIP-produced ^{123}I (decay of ^{123}Xe precursor in the ampoule, evacuation, rinsing out with dilute NaOH , NaHCO_3 , water, phosphate buffer pH 7, or many other solvents) consistently contained >95% I^- . Less than 5% of unidentifiable species (in addition to 0-0.5% iodate) were often present, but these did not significantly affect subsequent radioiodination reactions.

Iodination of IAMP was found to be somewhat sensitive to iodate contaminant in radioiodide solutions. In contrast, ^{123}I -HIPDM was obtained in high labeling yields (95-99%) even when considerable amounts of iodate were present in ^{123}I solutions. It appears that iodate is reduced to exchangeable iodide under the conditions of this iodination reaction (pH 2, 95°, excess HIPDM ligand) but not under the iodination conditions for IAMP. The effect of pH on the HIPDM labeling reaction was also followed by HPLC: at pH 2, only one major product peak was seen whereas at pH 3.6, 2-5% of an additional product was formed. These results correlated well with TLC analyses. Iodination of IAMP was also monitored by HPLC. It was possible to follow this reaction in order to optimize formation of the desired product under various conditions. Purification of IAMP following radioiodination also

appears feasible using the HPLC technique. In the case of ^{123}I IUDR, HPLC provided good separations on a C_{18} reverse-phase column using phosphate buffer, pH 5, in 10% methanol. The elution factors were as follows: 0 (uracil); 0.40 (UDR); 1.30 (iodouracil); and 2.50 (IUDR). Unbound iodide eluted much later with considerable tailing.

Results from these studies thus demonstrate the effectiveness of HPLC in allowing a fast and reliable separation not only of various chemical forms of iodine but of a number of iodinated radiopharmaceuticals as well. The developed methods provide excellent resolution and appear superior to the commonly used paper, thin-layer, and other chromatographic techniques^(7,17).

That the BLIP-produced ^{123}I is entirely suitable for radiiodinations and gives satisfactory radiopharmaceutical products has repeatedly been demonstrated by a number of users of this product. Typical results from our laboratory on the biological distribution of ^{123}I -labeled IAHP and HIPDM are described in Table 2. It is readily appreciated that the tissue distribution of these compounds in mice is, within statistical error, almost identical to that from ^{131}I -labeled analogs. We have recently labeled a number of monoclonal antibodies with BLIP-produced ^{123}I and demonstrated their immunoreactivity and biodistribution to be similar to analogous ^{131}I -labeled products^(18,19).

Acknowledgments

The labeling procedures and ligands used in this study were kindly provided by Medi-Physics, Inc. (iodoamphetamine), and H. Kung of SUNY, Buffalo, NY (HIPDM). The contributions of P. Richards in the earlier phases of this research are acknowledged. P. Som and K. Yamamoto assisted with the biodistribution studies in mice. Thanks are due to Mrs Rae Bailey for her help in the various stages of preparation of this manuscript. We also thank S. Katcoff and A. Wolf for making the CLIF available to us. This research was supported by the U.S. Department of Energy under Contract No. DE-AC02-76CH00016.

Legends for Figures

Figure 1. Schematic of gas train for separating ^{123}I from NaI target material

Figure 2. Design of test target for continuous flow production of ^{123}I

Figure 3. HPLC fractionation of a freshly prepared synthetic mixture containing iodate, iodide, and periodate. Column: RPS Lichrosorb 4.6 x 250 mm. Eluting buffer: 0.05 M phosphate and 0.002 M tetrabutylammonium hydroxide in 20% methanol, pH 7. Temp. 22°, flow rate 1 ml/min.

Figure 4. HPLC elution diagram of a BLIP-produced ^{123}I sample, following oxidation with NaOCl in base. Column: Lichrosorb RPS, 4.6 x 250 mm. Eluting solution: 0.05 M phosphate, 0.002 M tetrabutylammonium hydroxide, pH 7. At indicated point (9 min), the eluting solution contained 10% methanol in addition to the above components. Note the formation of iodate, and species x, y, and z of unknown composition, all presumably at the expense of I^- . Temp. 22°, flow rate 1 ml/min.

Figure 5. HPLC elution diagram of a BLIP-produced ^{123}I sample, following oxidation with NaOCl at pH 2. Fractionation conditions were the same as in Figure 4. Note the intense iodate peak, disappearance of I^- and peak y, and an increase in x.

Legends for Figures (contd.)

Figure 6. HPLC separation of a ^{123}I sample from another manufacturer. Eluting solution was 10% methanol containing 0.05 M phosphate and 0.002 M tetrabutylammonium hydroxide, pH 7. All other conditions were the same as in Figure 3. The BLIP-produced sample in comparison showed >95% iodide and <1% iodate.

REFERENCES

1. DeNardo G. L., Krohn K. A., Jansholt A. L. et al.
IAEA-SM-210/308, 3 (1977); Richards P., Lebowitz E. and Stang
L. G. Jr. IAEA-SM-171/38, 325 (1973).
2. Mausner L. F. and Richards P. IEEE Trans. Nucl. Sci.
NS-30, 1793 (1983); Stang L. G. Jr, Prog. Nucl. Med. 4, 34
(1978).
3. Richards P., Prach T., Srivastava S. C., and Meinken G. E.
J. Radioanal. Chem. 65, 47 (1981).
4. Srivastava S. C., Meinken G. E., Prach T., Mausner L. F., and
Richards P. J. Labeled Compds. Radiopharm. 21, 1135 (1984).
5. Katcoff S., Cumming J. G., Godel J., Buchanan V. J., Susskind
H., and Hsu C.J., Nucl. Instr. Meth. 129, 473 (1975).
6. Sandell E. B. and Kolthoff I. M. J. Amer. Chem. Soc. 56, 1426
(1934); ibid., Mikrochim. Acta 1, 9 (1937); Zak B., Willard
H. H., Meyers G. B., and Boyle A. J. Anal. Chem. 22, 939
(1950).
7. The United States Pharmacopeia, 19th revision (Mack Printing
Co., Easton, PA, 1974) p. 462; Mattson S., Persson R. B. R.,
Int. J. Appl. Radiat. Isotopes 27, 319 (1976); Procedure
Manual: Radiochemical Purity of Radiopharmaceuticals Using
Gelman ITLC Chromatography, Technical Bulletin No. 32,
(Gelman Instrument Co., Ann Arbor, MI, June 1977).
8. Carlsen L., Andresen K. Eur. J. Nucl. Med. 7, 280 (1982).
9. Kung H. F., Trampusch K. M., Blau M. J. Nucl. Med. 24, 66
(1983).

10. Syme D. G., Wood E., Blair I. M., Kew S., Perry M., and Cooper P. Int. J. Appl. Radiat. Isotopes 29, 29 (1978).
11. Diksic M. and Yaffe L. J. Inorg. Nucl. Chem. 39, 1299 (1977).
12. Paans A. M. J., Vaalburg W., van Herk G., and Woldring M.G. Int. J. Appl. Radiat. Isotopes 27, 465 (1976).
13. Wilkins S. R., Shimose S.T., Hines M. H., Jungerman J. A., Hegeclus F., and DeNardo G.L. Int. J. Appl. Radiat. Isotopes 26, 279 (1975).
14. Janni J. F. Air Force Weapons Laboratory Report AFWL-Tr-65-15 (1966).
15. Williamson C. F., Boujot J. P., and Picard J. Commissariat a l'Energie Atomique Report CEA-R3042 (1966).
16. Richards P. and Ku T. H. Int. J. Appl. Radiat. Isotopes 30, 250 (1979); Mausner L. F., Prach T., and Richards P. In Radionuclide Generators, New Systems for Nuclear Medicine Applications, F. F. Knapp, Jr. and T. A. Butler, Eds., (ACS, Washington, DC, 1984), pp. 77-95.
17. Rössler K., Tornau W., Stöcklin G. J. Radioanal. Chem. 21, 199 (1974).
18. Oster Z. H., Srivastava S. C., Som P., Meinken G. E., Scudder L. E., Yamamoto K., Atkins H. L., Brill A. B., and Collier B. S. Proc. Nat. Acad. Sci. U.S.A. 82, 3465 (1985).
19. Srivastava S. C. and Meinken G. E. In Radiochemical Labeling and Characterization of Proteins. (Springer-Verlag, New York, 1985. In press).

Table 1. Analysis of Various Radioiodine Species (k' values)^a by C₂, C₈ and C₁₈ Reverse-Phase High Performance Liquid Chromatography^b

Species	100% Aqueous			10% Acetonitrile			40% Acetonitrile		
	C ₂	C ₈	C ₁₈	C ₂	C ₈	C ₁₈	C ₂	C ₈	C ₁₈
IO ₃ ⁻	0.09	0	0	0	0	0	0	0	0
I ⁻	1.55	4.86	3.29	0.73	1.21	0.43	0.09	0	0
IO ₄ ⁻	11.2	-	R ^c	3.55	5.36	6.57	1.36	-	0
I ⁺	-	-	-	-	7.00	-	-	-	-
CH ₃ I	1.54	-	3.71	1.55	4.14	2.86	1.0	1.57	1.0
CHI ₃	R	-	R	R	R	R	3.0	10.7	3.71

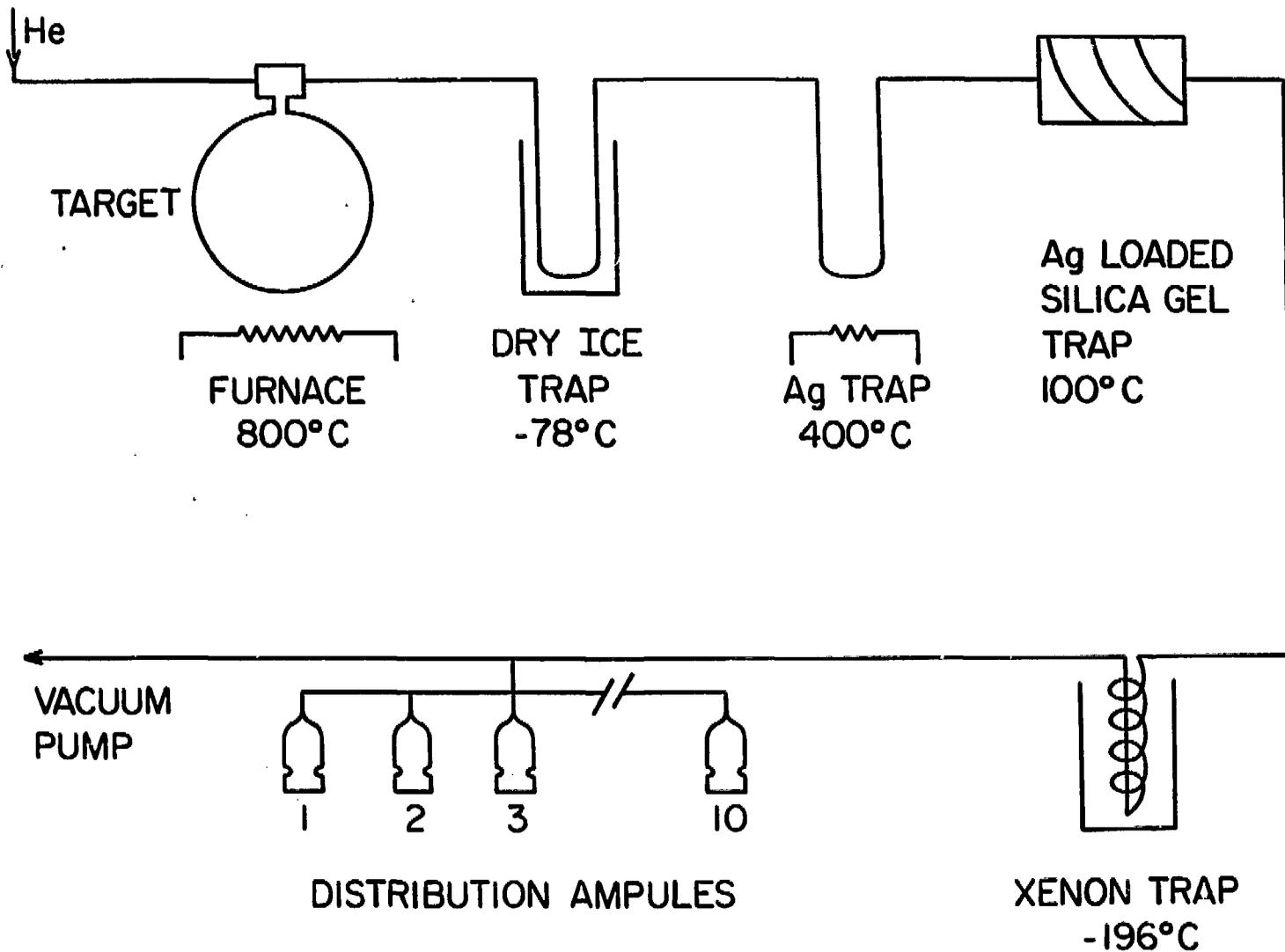
^a $k' = \frac{\text{elution volume (V}_e\text{)} - \text{column volume (V}_o\text{)}}{V_o}$

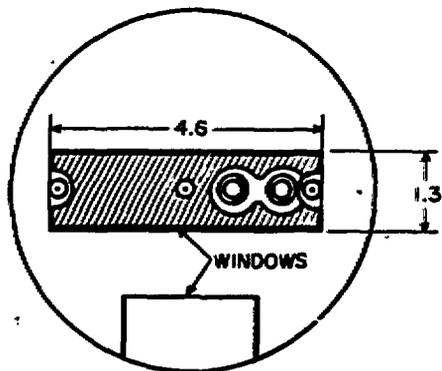
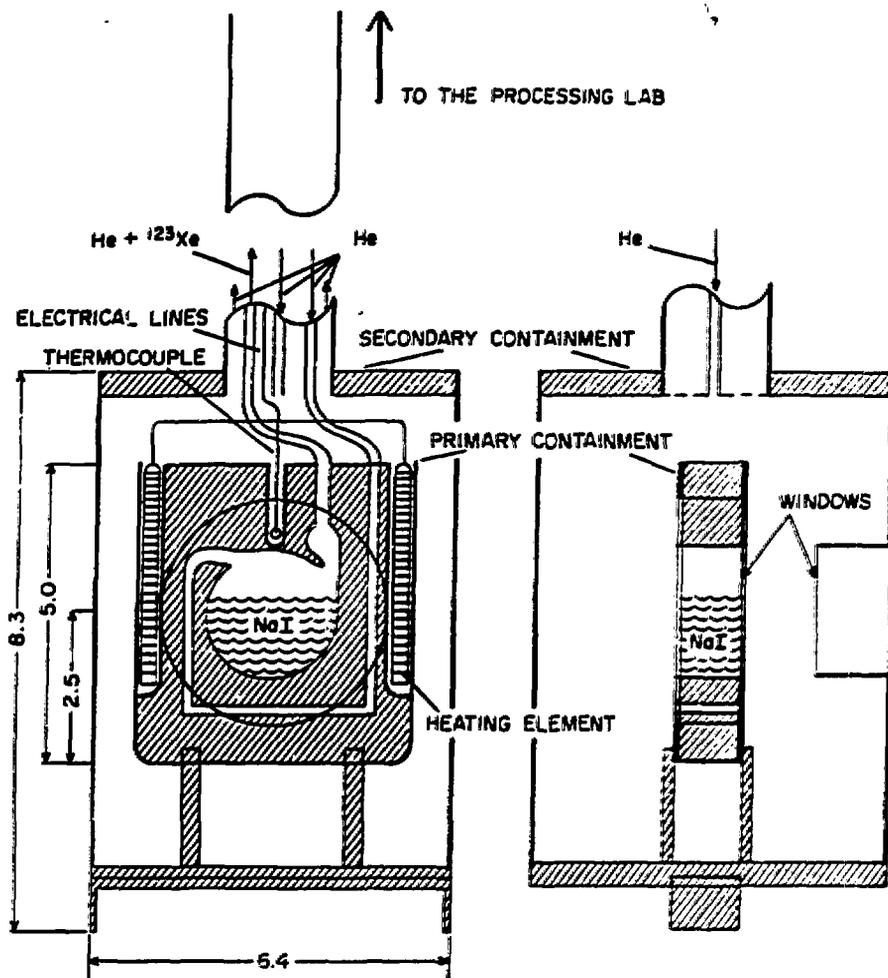
^bElution buffer was a 0.05 M pH 7 phosphate solution containing the stated amount of aqueous or organic phase and 0.002 M tetrabutylammonium hydroxide.

^cRetained

Table 2. Tissue Distribution in Mice (%Dose/Organ) of Radioiodinated IAMP and HIPDM, 60 Minutes Post Injection (n = 4)

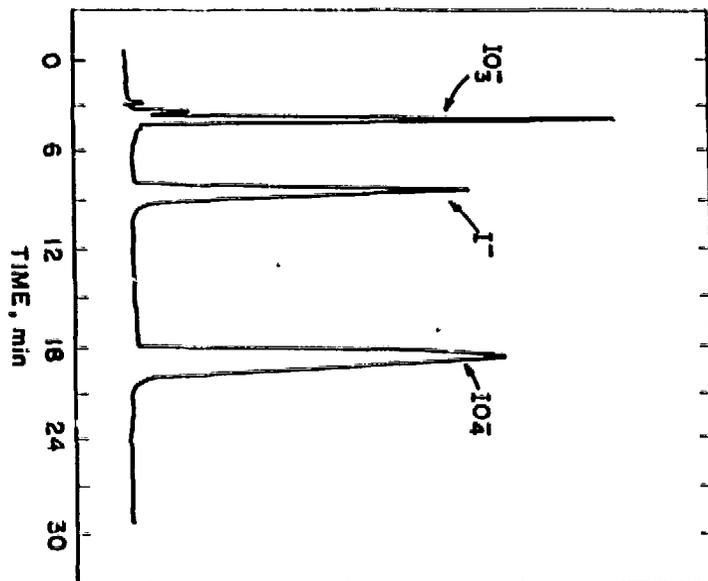
Organ	^{123}I -IAMP	^{131}I -IAMP	^{123}I -HIPDM	^{131}I -HIPDM
Blood	2.02 ± 0.48	3.01 ± 0.44	1.29 ± 0.12	0.80 ± 0.07
Brain	3.64 ± 0.30	4.26 ± 0.50	4.47 ± 0.28	4.21 ± 0.34
Lungs	2.86 ± 0.31	3.66 ± 0.64	3.01 ± 0.95	3.87 ± 0.74
Liver	17.50 ± 1.02	13.92 ± 2.41	16.57 ± 2.12	13.42 ± 0.77
Kidneys	3.94 ± 0.37	5.46 ± 1.04	3.97 ± 0.18	3.72 ± 0.07
Spleen	2.21 ± 0.14	0.84 ± 0.23	1.41 ± 0.12	1.38 ± 0.10
Gut and Feces	15.90 ± 0.22	15.22 ± 0.77	19.17 ± 0.62	18.91 ± 0.22



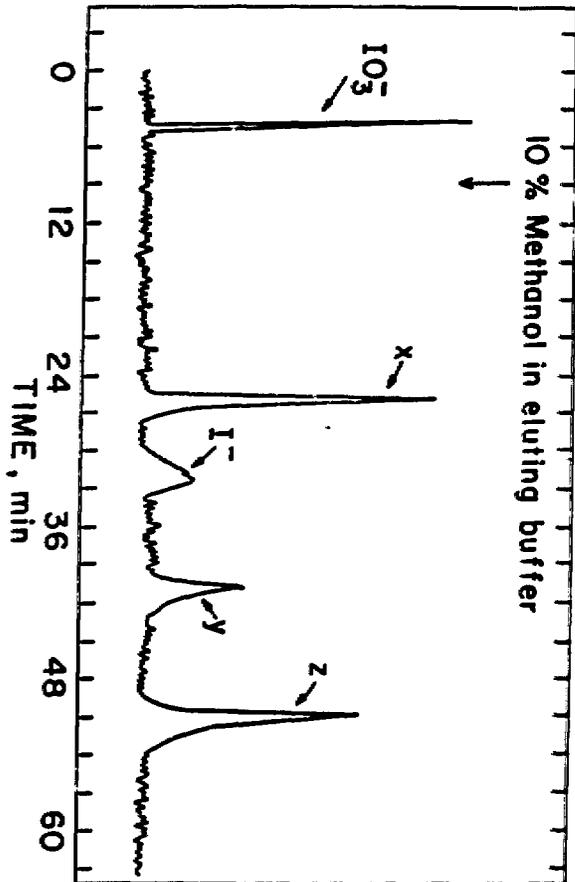


UNITS ARE GIVEN IN cm

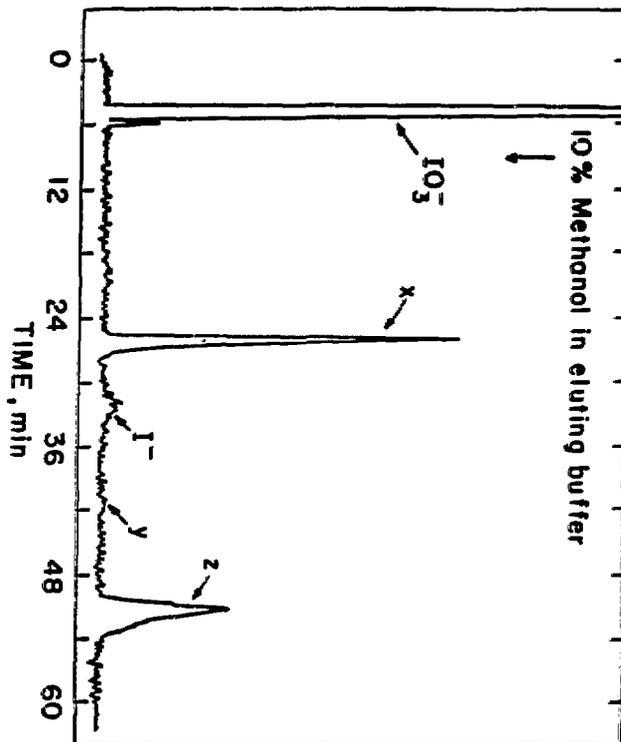
ABSORBANCE, 225 nm



IODINE-123 ACTIVITY



IODINE-123 ACTIVITY



IODINE-123 ACTIVITY

