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PERACETIC ACID AS A SUPERIOR OXIDANT FOR PREPARATION OF [^{123}I]IBZM: A POTENTIAL DOPAMINE D-2 RECEPTOR IMAGING AGENT

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Summary

Various oxidizing agents: chloramine-T, hydrogen peroxide, sodium persulfate, m-chloroperoxybenzoic acid and peracetic acid were examined as the oxidant for preparing radioiodinated IBZM ((*S*)-(-)-3- iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide), which is a useful dopamine D-2 receptor imaging agent. Of all the oxidizing agents tested, peracetic acid appears to be the best agent for no-carrier added radioiodination. The advantages of using peracetic acid as the oxidant for the preparation of [^{125}I] or [^{123}I] IBZM include: high radiochemical yield, high radiochemical purity, and short reaction time at room temperature.

Key Words: radioiodination, oxidants, IBZM, peracetic acid, chloramine-T, hydrogen peroxide

Introduction

Radioactive iodinated compounds, either labeled with I-125 ($T_{1/2}$ = 60 days, gamma ray 30-65 Kev), I-131 ($T_{1/2}$ = 8 days, gamma ray 364 Kev) or I-123 ($T_{1/2}$ =13 hr, gamma ray 159 Kev), are tracers for radioimmunoassay (RIA), receptor binding assays and nuclear medicine imaging. The I-125 labeled compounds are useful for *in vitro* studies, while I-123 labeled compounds, which emit a superior gamma ray (159 Kev) for external detection with a gamma camera, are better suited for *in vivo* imaging. There are two ways of preparing radioactive iodinated compounds: by an iodine-iodine exchange reaction or by an oxidative iodination. The major attractive feature of the radioactive iodination is that the iodinated

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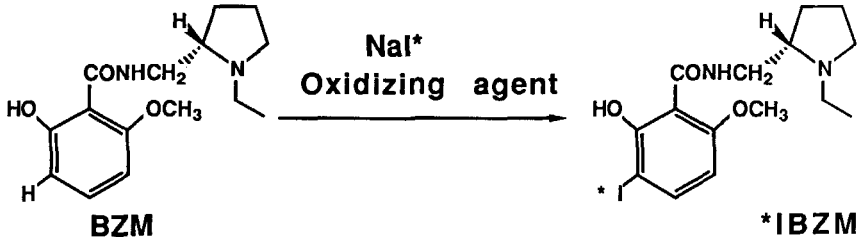
compound produced by this method is of high specific activity. Radioactive agents with higher specific activity are better suited as tracers for *in vitro* and *in vivo* evaluation of specific binding sites. When radioactive iodinated compounds are produced at a no-carrier added level, theoretical specific activities for I-125 and I-123 compounds are 2.2×10^3 and 2.4×10^5 Ci/mmole, respectively.

The majority of oxidative radioiodination procedures was originally developed for radiolabeling proteins.[1,2] The active iodinating moiety is generated by oxidizing I^- to I^+, by which the electrophilic oxidative iodination of phenols and other activated ring systems takes place.[3,4] Methods for radiolabeling small molecules have been reviewed thoroughly by Counsell.[5] Chloramine-T (CT) is commonly used as the oxidizing agent for both - radiolabeling of proteins and small molecules. Due to its strong oxidation potential, this oxidant is prone to producing side reactions. Radiolabeling reactions involving CT normally require a fine control of molar ratio of oxidant to substrate, and reaction time (in seconds to a few minutes). In addition, the CT reaction often produces chlorinated side products which decrease radiolabeling yield and are difficult to separate from the desired iodinated compounds. A number of other oxidants for radioactive iodination have been reported - these include: hydrogen peroxide,[6] ammonium persulfate,[7] nitric acid,[8] iodate,[9] and chlorine water,[10] etc.

IBZM ((*S*)-(-)-3-Iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2- pyrrolidinyl)methyl]benzamide) (Scheme 1) belongs to a group of structurally related benzamides, which display significant antidopaminergic activity.[11] The *in vivo* and *in vitro* studies indicated that IBZM binds specifically to the CNS dopamine D-2 receptor with high affinity (Kd = 0.43nM) and stereospecificity.[12-14] The preliminary imaging studies done recently on monkeys and humans clearly demonstrated that IBZM is localized in the basal ganglia which contain high concentrations of D-2 receptor.[15] In developing [^{123}I]IBZM as a CNS D-2 dopamine receptor imaging agent, several oxidants (hydrogen peroxide, peracetic acid, m-chloroperoxybenzoic acid and sodium persulfate) were evaluated as alternatives to CT to improve the radioactive iodination reaction for preparing the [^{123}I]IBZM at a no-carrier added level. Since the final product, no-carrier added [^{123}I]IBZM, is to be injected into humans, stringent requirements are placed on the radioiodination procedure. The desirable characteristics for radioiodination are high yield, requiring a short reaction time and a simple purification process. At the end of preparation the product will have to be formulated under a sterile and pyrogen free condition. Taking all of these factors into consideration, various oxidants were studied for preparing [^{123}I]IBZM by the radioiodination reaction. For convenience, most of the studies reported in this paper were done with I-125; however, all of the results are valid when I-123 is employed instead of I-125.

Scheme 1 Radioiodination of BZM to IBZM



Experimental

Reagents

The uniodinated starting material, BZM ((*S*)-(-)-2-hydroxy- 6-methoxy-*N*-[(1-ethyl-2- pyrrolidinyl)methyl]benzamide), was prepared by a method described previously.[12] Sodium [^{125}I]iodide was obtained from Amersham in a no-carrier added form (specific activity 17Ci/mg; 2200 Ci/mmole). Sodium [^{123}I]iodide was obtained from Atomic Energy of Canada Ltd. (specific activity 2.4x10^{5}Ci/mmole). Chloramine-T hydrate (CT), peracetic acid (32 wt% solution in dilute acetic acid), sodium persulfate (98%) and m-chloroperoxybenzoic acid (80-85%) were purchased from Aldrich Chemicals. The m-chloroperoxybenzoic acid was repurified by washing with phosphate buffer (pH 7.4) and filtered to remove the impurity - benzoic acid. The purified material was dried under vacuum to give a sample with >95% purity. Peracetic acid was diluted from stock solution (32% wt) with distilled water before use. Hydrogen peroxide (30%) was obtained from Fisher Scientific and diluted to 3% with distilled water before use. Ethyl acetate and acetonitrile were of HPLC grade and purchased from J.T. Baker. All other chemicals were of reagent grade and purchased commercially.

Radiolabeling

A desired quantity of oxidizing agent (in a volume of 100ul) was added to a mixture of BZM (0.18 umole in 50 ul EtOH), sodium [^{125}I] or [^{123}I]iodide (10 ul, 20-50 uCi), and buffer (0.3 ml, sodium phosphate, pH 3.0 or ammonium acetate, pH 4.0) solution in a sealed vial (total volume was 0.45ml). The reaction was allowed to proceed at room temperature, 65°C or 100°C for a specific time period. The oxidation reaction was terminated by the addition of an excess amount of reducing agent - sodium bisulfate (0.1ml, 200mg/ml) and neutralized with saturated sodium bicarbonate (0.5-1.0ml). The product was either analyzed directly by TLC or by solvent extraction (ethyl acetate,1mlx3). The combined organic layers were dried by being passed through an anhydrous sodium sulfate column (0.2cmx5cm). The radiolabeling yield was determined by dividing the radioactivity associated with the ethyl acetate layer by total activity. The organic

solution was then condensed under a stream of nitrogen and the residue was dissolved in absolute ethanol (10-20ul). The radiochemical purity was analyzed by HPLC as described below. The various amounts of BZM and the pH values used for the iodination reaction were studied in separate experiments to measure their effects on radiolabeling yield.

Radiochemical purity analysis

The radiochemical purity was determined by thin-layer chromatography (TLC) using silica gel plates (60 F-254, Merck) developed by a solvent system, namely - $CHCl_3$:C_2H_5OH: con.NH_3 (9:1:0.2). The authentic "cold" IBZM standard gave an R_f value of 0.7-0.8, whereas the free iodide (I^-) stayed at origin (R_f=0). At the end of solvent development, the chromatograms were dried and cut into 5 mm fractions and counted in a Beckman gamma counter. The purity study on HPLC was performed on a reverse phase column (PRP-1, Hamilton), and it was eluted with an isocratic solvent system - acetonitrile: 5mM 3,3'-dimethylglutaric acid, pH 7.0 (82:18) in a flow rate of 1 ml/min. The percentage of the radiochemical purity was calculated by dividing the total integrated counts of the desired peak with a retention time of 15 min (corresponding to IBZM) to total integrated counts.

Results and Discussion

Preparation of radioactive IBZM by a radioiodination reaction was studied using various oxidizing agents. Most of the results were from reactions using I-125 isotope, however similar reaction conditions can be applied on the same reaction using I-123 isotope. The labeled product obtained was compared with chemically pure nonradioactive IBZM on HPLC using simultaneous gamma and u.v. detection, respectively. Based on the elution profiles, it is clearly demonstrated that the radioiodinated IBZM displays the same retention time (~15 min) as that of the authentic sample (cold IBZM, detected by u.v. tracing); whereas the uniodinated starting material, BZM, under the same chromatographic conditions, showed a retention time of 8 min. Two HPLC profiles are shown in Fig. 1. When hydrogen peroxide (H_2O_2) or peracetic acid was used as the oxidizing agent, the radioiodinated product (profile A, Fig 1) displayed high radiochemical purity. The majority of the radioactivity was associated with the peak (measured by gamma detector) showing the same retention time as that of the "cold" IBZM peak (measured by UV detector). When other oxidizing agent, such as m-chloroperoxybenzoic acid or sodium persulfate, was used, the radioiodinated product (profile B of Fig 1) exhibited lower radiochemical purity. In addition to the desired [^{125}I]IBZM, there are other minor impurities eluted prior to the major peak.

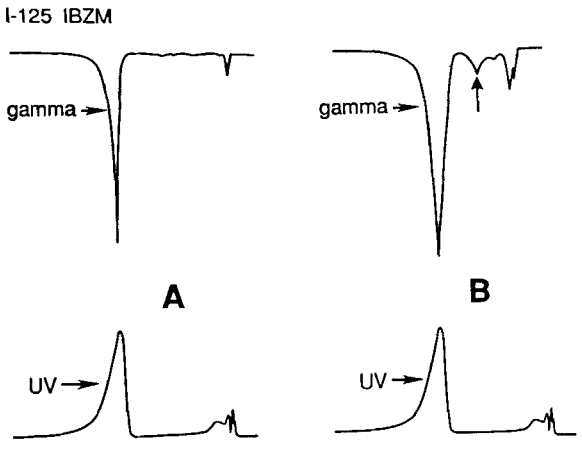


Fig. 1. Two high pressure liquid chromatography profiles of carrier-free [^{125}I]IBZM (gamma) and carrier added IBZM (u.v.):
Profile A: a preparation with high radiochemical purity;
Profile B: a preparation contaminated with undesired side products.

The optimum conditions for radioiodination of BZM, with various oxidizing agents, are summarized in Table 1. The radiolabeling reaction of BZM with CT was instantaneous at room temperature. The optimum condition for radiolabeling was at pH 3.0 with BZM (0.18 umole) to CT (0.22 umole) in 0.3 ml buffer (Table 1). The total radiolabeling yield was 80-90%.

Table 1 Optimum radioiodinating conditions with various oxidizing agents

Oxidizing agent	Amt. (umole)	Buffer	Temp (°C)	Rx. time (min.)	Labeling yield(%)	Radiochem. purity	n
chloramine-T	0.22	Na-phosp pH 3.0	RT	1.5	80-90	90-95	10
hydrogen peroxide	82.00	Na-phosp pH 3.0	100°C	30.0	85-90	92-95	10
peracetic acid	4.20	NH_4OAc pH 4.0	RT	2.0	90-95	93-95	10
m-chloroperoxy-benzoic acid	0.29	Na-phosp pH 3.0	65°C	30.0	70-79	85-90	3
sodium persulfate	420.00	Na-phosp pH 3.0	65°C	30.0	35-40	65-68	3

A constant amount of BZM (0.18 umole) was used for iodination (total volume 0.45 ml). Sodium [^{125}I]iodide was used for the study.

However, the molar ratio of the oxidant (CT) to the substrate (BZM) showed a significant effect on the radiolabeling yield (see Table 2) and the iodination reaction with CT preferred acidic pH (pH 3.0) (data not shown). When the reaction was allowed to proceed for more than 2 min., side product(s) started to appear (data not shown). Thus, in order to avoid the possibility of undesired side reactions, the reaction time with CT was kept short (1.5 min.).

Table 2 Effect of molar ratio of chloramine-T to BZM on radiolabeling yield

• molar ratio(CT/BZM)	% radiolabeling yield	% radiochem. purity
1.2	92	96.4
4.1	90	70.0
12.2	90	65.0

Iodination reaction was carried out at room temperature for 1.5 min at pH 3.0 phosphate buffer (total volume 0.45 ml).

Hydrogen peroxide gave high radiolabeling yield (85-90%) and high radiochemical purity (92-95%) as indicated in Table 1. Radiolabeling with hydrogen peroxide only proceeds well at a higher temperature (100°C) and prolonged reaction time (20-30 min). Similar to CT, acidic pH values were preferred. However, a wider range of ratio of substrate (BZM) to oxidant (H_2O_2) concentration, which gives optimum radiochemical yield, is superior to that of CT (Table 3).

Table 3 The effect of temperature and hydrogen peroxide concentration on radiolabeling yield*

amt of H_2O_2 (umole)	temp(°C)	pH	Radiolabeling yield
16	100	3.0	90.0
41	100	3.0	90.7
82	100	3.0	90.4
16	25	3.0	41.5
41	25	3.0	55.4
82	25	3.0	43.9
41	100	3.0	90.7
41	100	4.0	81.3

*The results were similar under two concentrations of BZM - 0.180 umole and 0.018 umole in a total volume of 0.45 ml.

Meta-chloroperoxybenzoic acid (CPA) is the oxidant which had not been studied previously for radioiodination. As shown in Table 1, the optimum radiolabeling condition for CPA is at 65°C and under acidic pH (pH 3.0). The reaction requires prolonged heating (~30 min). The radiolabeling yield and radiochemical purity were 70-79% and 85-90%, respectively.

Preparation of radiolabeled IBZM has also been examined using sodium persulfate as the oxidant. As shown in Table 1, the radiolabeling reaction did not proceed well and the reaction produced undesired side-products. Due to the instability of the oxidant (sodium persulfate) in aqueous solution and the elevated temperature, this oxidant is the least suitable for radioiodination.

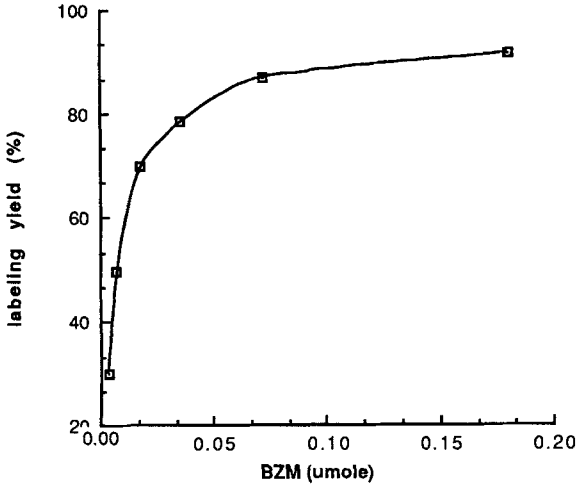


Fig. 2. The effect of substrate (BZM) concentration on the radiolabeling yield under a constant amount of peracetic acid (0.42 umole) and a fixed reaction time and temperature (1 min., RT).

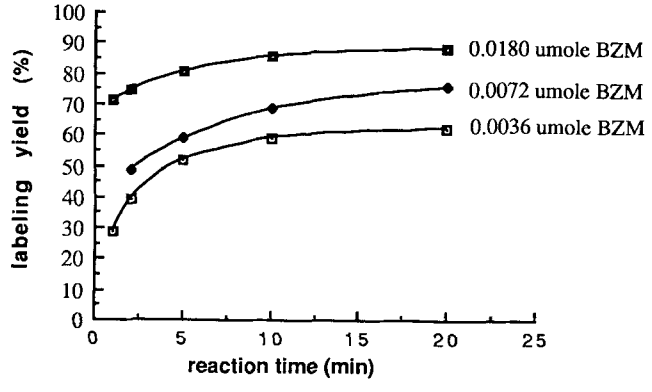


Fig. 3. Radiolabeling yield of [^{125}I]IBZM as a function of reaction time at three different substrate concentrations in the presence of a constant amount of peracetic acid (0.42 umole), and the total volume of the solution was 0.45 ml.

Of all the oxidants studied (Table 1), peracetic acid was the best for radiolabeling of IBZM, giving high yield (92-95%) and pure product (93-95%). It only requires a short reaction time (2 min.) and low temperature (25°C) and is superior to all of the other oxidants. Furthermore, radiolabeling yields of iodination with peracetic acid were examined as a function of substrate (BZM) concentration. As the concentration of BZM increases, the radiolabeling yield increases (Fig. 2). As expected for a second order reaction, the iodination reaction occurred faster with higher substrate (BZM) concentration and the radiolabeling yield reached more than 70% at the highest BZM concentration studied (0.04mM) (Fig. 3). The concentration effect of peracetic acid and the effect of reaction time on the total yield were also studied. As shown in Table 4, there is a wide range of substrate (BZM) to oxidant (peracetic acid) ratio. Iodination performed with peracetic acid at different time points also indicated that a prolonged reaction time does not give any undesired product (see Table 5). These characteristics associated with peracetic acid are unique, and therefore this agent is superior to the other oxidants studied. Similar iodination conditions, as those for I-125, have been successfully applied on I-123 isotope for the preparation of [^{123}I]IBZM. The simple and no side-product iodination reaction using peracetic acid is the best procedure for preparing [^{123}I]IBZM for imaging study. Due to the limited sensitivity of UV detectors, it was reported previously that the specific activity of [^{125}I] IBZM was 600 Ci/mmole.[14] It is expected that [^{123}I]IBZM will have much higher specific activity; for a carrier-free preparation the value is 2.4 x 10^5Ci/mmole. Based on the preparation procedure described in this paper, it is reasonable to assume that the preparation is most likely carrier-free, which is highly desirable for CNS dopamine receptor imaging in humans.

Table 4 Effect of peracetic acid concentration on radiolabeling yield

Amt. of peracetic acid (umole)	radiolabeling yield (%)
0.21	90.5
0.42	91.1
1.31	>95.0
2.62	>95.0
10.52	>95.0
21.05	>95.0
42.10	95.0

Radioiodination was performed at room temperature for 1 min. with 0.18 umole of BZM in a total volume of 0.45 ml.

Table 5 Effect of reaction time on radiolabeling yield with peracetic acid

Time (min.)	radiolabeling yield (%)
1	95
2	94
5	90
10	95
20	92

The reaction was carried out at room temperature with 0.18 umole of BZM and 4.21 umole of peracetic acid in a total volume of 0.45 ml.

An earlier study reported in the literature, on the nonradioactive iodination of benzene and phenyl compounds with a mixture of peroxyacetic acid and iodine, showed an excellent chemical yield.[16] While this work was in progress, a paper was published describing no-carrier added radiohalogenation ([^{77}Br]bromine and [^{131}I]iodide) using *in situ* generated peracetic acid.[17] Our results on the preparation of [^{125}I]IBZM confirm the usefulness of peracetic acid as an oxidizing agent at no-carrier added level. Using peracetic acid diluted directly from a commercially available source appears to be advantageous.

Under the same condition, peracetic acid is also found to be a superior alternative oxidizing agent for the preparation of iodinated benzapepine derivatives: R-(+)-8-[^{123}I]iodo-2,3,4,5- tetrahydro-3-methyl-5-phenyl-1H-3- benzapepine- 7-ol ([^{123}I]IBZP), a potential dopamine D-1 receptor imaging agent. While our work was in progress, a similar observation on the utility of peracetic acid as an oxidant for the preparation of [^{125}I]IBZP was reported.[18] Radioiodination of protein molecules with peracetic acid is also currently under investigation.

In conclusion, peracetic acid is the best oxidizing agent for the preparation of [^{123}I] and [^{125}I]IBZM; the optimal iodination conditions performed are at room temperature for 2 min in ammonium acetate buffer (pH 4.0) with BZM (0.18 umole) to peracetic acid (4.2 umole). The radiolabeling reaction carried out in the presence of this oxidant is rapid, efficient and produces no side products. It is likely that this oxidant will be applicable to other oxidative radioiodination reactions of different types of organic molecules.

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