Applied Radiation and Isotopes 68 (2010) 309–313

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

The Cu^+-assisted radioiodination *Kit*: Mechanistic study of unexplored parameters concerning the acidity and redox properties of the reaction medium

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ARTICLE INFO

Article history:
Received 17 February 2009
Received in revised form
7 October 2009
Accepted 7 October 2009

Keywords:
Radioiodination
MIBG
[^{123}I] labeling

ABSTRACT

Nucleophilic Cu^+-assisted radioiodination can be optimally performed at pH ~ 2.3 by using conventional reducing agents such as gentisic acid and $SnSO_4$, mixed or separately.

A mechanistic overview of the Cu^+-radioiodination method is presented in the extended pH-range of 1–4.4. At lower pH, these usual reducing agents show a distinct behaviour. Oxidizing acids (HSO_4^-, H_3PO_4) must be avoided, where as redox neutral acids (trifluoroacetic acid or methanesulfonic acid) or reducing acids (H_2SO_3, H_3PO_2) are well tolerated.

The presence of reducing acids makes the use of the usual reducing agents redundant.

1. Introduction

The well-established Cu^+-assisted nucleophilic radioiodination method in acid reducing conditions has been successfully applied for the radiolabeling of different types of radiopharmaceuticals (Coenen et al., 2006a). Cu^+-assisted nucleophilic radioiodination can be conducted via non isotopic exchange (*I/Br) and by isotopic exchange in water as well as in mixed solvents, i.e. fatty acids (Mertens et al., 1987), iomazenil (Eersels et al., 2005) and amino acids (Kersemans et al., 2006; Bauwens et al., 2006). A first mechanistic approach of the method has been described by Mertens and Gysemans (1991). The use of non-toxic auxiliary materials in the reaction mixture (i.e. reducing agents and complexing agent), as well as the potentiality of quantitative labeling and the absence of non radioactive and/or radioactive side products offer the opportunity for a "*Kit*"-development of iodinated radiopharmaceuticals. The reliability of this method proves its use not only in small laboratory scale preparations, but also in manufacturing production processes of ^{123}I-labeled radiopharmaceuticals (Eersels et al., 2005).

The aim of this study is a mechanistic evaluation of the influence of the acidity and the effect of the redox properties of inorganic and organic acids that can be used in the reaction mixture for purposes of solubility or finalization of the pharmaceutical composition like phosphate buffer.

In addition, modified reaction conditions are proposed, which can offer an alternative strategy for the standard reaction conditions.

In this study the radiopharmaceutical meta-iodobenzylguanidine (MIBG) is used as reference molecule for radiolabeling.

2. Materials and methods

2.1. Reagents

All reagents and solvents were obtained from commercial suppliers and were HPLC- or analytical grade and used as such. Hypophosphorous acid (H_3PO_2) was purchased in a 50 wt%-solution in water (9.45 M) and 2,9-dimethyl-1,10-phenanthroline (neocuproïne) in its free-base form.

MIBG sulfate was donated by Mallinckrodt Medical, The Netherlands.

Nitrogen was 5.0-grade and purchased from Hoekloos, The Netherlands.

Radioiodide (Na[^{123}I], no carrier added; specific activity of 8695 GBq/μmol) in 10^{-2} M NaOH was obtained from BV Cyclotron Vrije Universiteit Amsterdam.

2.2. HPLC equipment and analyses

Control of the radiochemical purity of the $^{123}I^-$ was performed by means of ion-pair chromatography, as earlier described (Eersels et al., 1995; Vanryckegem and Mertens, 1989).

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doi:10.1016/j.apradiso.2009.10.009

The reaction mixture for the labeling of MIBG with ^{123}I was analyzed by HPLC: Rheodyne injector (0.2 ml loop), a LKB pump with a Jasco UV monitor at 230 nm, a flow-through NaI(Tl)-radioactivity detector (Ortecs electronics), a RP Select B, LiChrosorb (Merck) column, 125 × 4 mm, 5μ with a MeOH/H_2O eluent, 0.08 M sodium dihydrogenphosphate solution 35/65 (pH ~ 4.9) at a flow rate of 0.95 ml/min.

All chromatographic data were filed and analyzed using Gina Star software, version 14.0.

2.3. Labelingprocedure and experiments

- Radioactivity: Radioactivity was measured using a Veenstra dose calibrator, type VDC 404.
- Radioiodination: Labeling experiments were carried out in a 2 ml flat-bottom vial (with PTFE-faced silicone septum and open top crimp cap) with weighed amounts of MIBG, citric acid, tartaric acid, $SnSO_4$ or 2,5-dihydroxybenzoic acid (gentisic acid). Afterwards 0.1 mg $CuSO_4$ was added from a stock solution (30 μl of a 13 mM $CuSO_4 \cdot 5H_2O$ in 10 ml water) and final volume was adjusted to 1 ml using distilled water. Subsequently, 10–20 MBq Na[^{123}I] (5–10 μl) was added, the vial was crimped and the content flushed with a gentle stream of N_2 during 15 min at room temperature.

The vial was placed in a copper container containing paraffin oil and heated in a thermoblock at 110 °C during 40 min. After fast cooling of the reaction mixture to room temperature, a sample was taken for HPLC analysis.

Influence of pH: Reaction mixtures of pH 0.9–1.1 were obtained by addition of an acid solution of appropriate concentration—0.5 M for $NaHSO_4$, 1 M for H_3PO_4, 0.11 M for trifluoroacetic acid (TFA), 0.1 M for methanesulfonic acid (MSA), 0.42 M for H_3PO_2 and 0.5 M for H_2SO_3—to the weighed amounts of MIBG, citric acid, tartaric acid, $SnSO_4$, gentisic acid and $CuSO_4$, to an end-volume of 1 ml, and further worked-up as described above.

Reaction mixtures with a pH > 2.5 were prepared by addition of a 5×10^{-2} M NaOH solution to the initial composition.

Variable concentrations of Sn^{2+} were obtained by adding appropriate amounts of a $SnSO_4$ solution in presence of complexing agents, i.e. citric and tartaric acid.

2.4. Experiments in non-radioactive conditions

- *Influence of the redox properties of the medium*: The reducing potency of respectively $SnSO_4$, gentisic acid and H_3PO_2 *vis-à-vis* $NaIO_3$ (I^{5+}) was measured in non radioactive conditions. The experiments were carried out with 1.6 redox-equivalents of reducing agent each (respectively 1 mg $SnSO_4$, 0.75 mg gentisic acid, and 1 ml of a 5 mM H_3PO_2 solution) and were added to three different vials, each containing 1 ml of a 1 mM aqueous $NaIO_3$ solution and 4 mg of citric acid. All reaction mixtures were brought to an end-volume of 2 ml with water, having a comparable pH of 2.5.
  Experiments were performed in oxygen-free conditions at 110 °C during 10 min. The reaction mixtures were analyzed after reaction by means of HPLC.
- *Prove of the 'in-situ' Cu^+-formation*: From a solution 3.2 mg of $CuSO_4 \cdot 5H_2O$ and 5.3 mg neocuproïne (2.54×10^{-2} M) in 1 ml of a 8/2 ethanol-water mixture, 30 μl was added to a standard labeling-reaction mixture containing 2 mg MIBG.1/2H_2SO_4, 5 mg gentisic acid, 6 mg citric acid and 0.6 mg $SnSO_4$ in 1 ml water (radioactivity was omitted).

3. Results

Standard conditions are referred as those used for radiohalogenation as earlier described (Eersels et al., 2005), i.e. 2 mg MIBG.1/2H_2SO_4, 5 mg gentisic acid, 6 mg citric acid, 0.6 mg $SnSO_4$ and 0.1 mg $CuSO_4$ in 1 ml water (pH of the mixture is ~2.3). The labeling yield obtained in these standard conditions amounts at least 99.0%.

The labeling yield is defined as the ratio of the amount of the labeled compound to the initial amount of activity calculated from the surfaces of the peaks in the radiochromatogram.

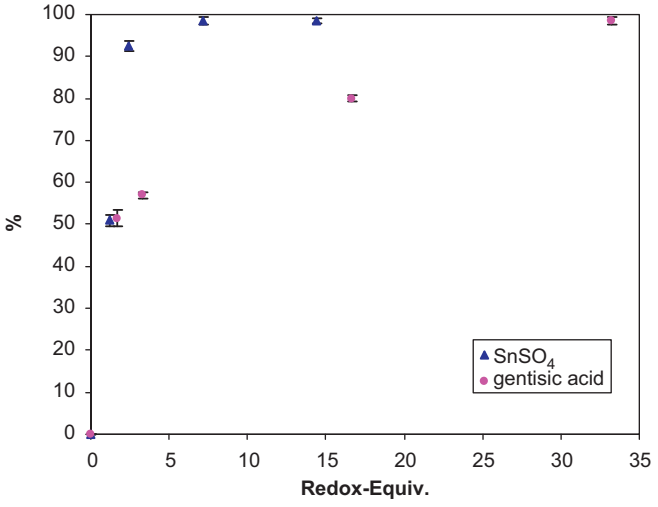
3.1. Comparison of the reducing potency of $SnSO_4$ and gentisic acid upon the labeling yield

Fig. 1 shows the influence of variable amounts of $SnSO_4$ or gentisic acid upon the labeling yield at pH ~ 2.3.

The two reducing systems show a completely different pattern. In the case of $SnSO_4$ an almost quantitative labeling using seven redox-equivalents ($Sn^{4+}/Sn^{2+}//Cu^{2+}/Cu^{1+}$) was achieved, while in case of gentisic acid the labeling yield slowly increases with the amount of the reductor and more than 30 redox-equivalents are required to reach the maximal yield.

3.2. Influence of pH upon the labeling yield

The reaction mixture of standard *kit* conditions was assessed at three different pH-values.



-values are represented as mean-values, n=5
-reaction mixture, performed with single reducing agent;
- *SnSO4* : 2 mg MIBG, X mg $SnSO_4$, 6 mg citric acid and 0.1 mg $CuSO_4.5H_2O$
- *gentisic acid* : 2 mg MIBG, X mg gentisic acid, 6 mg citric acid and 0.1 mg $CuSO_4.5H_2O$

Fig. 1. Labeling yield of MIBG vs. redox-equivalents for [$SnSO_4$]/[Cu^{2+}] and [gentisic acid]/[Cu^{2+}]-systems, at standard pH ~ 2.3.

Table 1
Influence of pH upon the labeling yield.

pH	Labeling yield (%)
1.0	84.9 ± 1.2
2.3	98.4 ± 0.4
4.4	98.6 ± 0.4

Values are represented as mean-values, *n*=5.
Reaction mixture: 2 mg MIBG, 0.6 mg $SnSO_4$, 5 mg gentisic acid, 6 mg citric acid and 0.1 mg $CuSO_4 \cdot 5H_2O$.