Synthesis of $^{131}$I Iopamidol as a Tracer for Development of Iopamidol CT-Scan Contrast Agent

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Abstract. Computed Tomography (CT) Scan is a means of imaging/diagnostic method which is preferable in hospitals in terms of availability, efficiency and cost. Currently, CT contrast agent most widely used is the iodinated derivatives, due to its high X-ray absorption coefficient. Contrast agent is a medical preparation used in CT-scan modality which has capability of enhancing the performance of CT-scan to differentiate between target organs and surrounding tissues, and one of them is iopamidol. Development of iopamidol contrast agent requires several steps, i.e formulation, characterization, in-vitro and in-vivo tests prior to clinical study. To do in vivo study or biodistribution study in experimental animals, a radioactive iopamidol should be used to trace the compound throughout the body to predict its pharmacokinetics, and for that purpose a radioiodine (Iodium-131 or $^{131}$I) labeled iopamidol will be used. Optimisation in the synthesis of $^{131}$I iopamidol was carried out by varying pH, temperature and reaction time to obtain $^{131}$I iopamidol with high radiochemical purity of more than 90% as a requirement of radiopharmaceutical preparation. The radiolabeled product was characterized using HPLC and the labeling efficiency was measured by TLC. The optimum condition obtained was repeated 3 times and the product was tested for stability in room temperature. Characterization using HPLC showed that retention time ($R_t$) of radiolabeled iopamidol was close to that of native iopamidol at ~ 6 min, indicating that $^{131}$I iopamidol was already formed. Iodium-131 labeled iopamidol has been successfully synthesized with labeling efficiency or radiochemical purity of 96% ± 1%, and the optimum condition of $^{131}$I iopamidol reaction was obtained at pH 9, temperature of 140°C in 5 minutes reaction time, and the product was stable in room temperature up to 7 days. It is concluded that radioiodinated iopamidol has been successfully synthesized and will be used in the formulation of iopamidol as a part of iopamidol contrast agent development for CT-Scan purpose.

1. Introduction

Computed Tomography (CT scan) is a popular radiodiagnostic modality that has been widely used in radiology. It works based on X-ray combined with a computer to perform an image of organ slices. To improve the image, CT scan is usually supported by the use of a contrast agent [1, 2]. Contrast media is radioopaque and radiolucent when interact with X-ray, so it can enhance the quality of an image generated by a CT scan in differentiating the target organ from the surrounding tissues [3].
There are various contrast media, one of which is an iodinated contrast media that has ionic and non-ionic properties, and the non-ionic contrast media which is commonly used is iopamidol. Iopamidol is administered to the patient through intravenous injection prior to examination with CT scan [4-7].

As a pharmaceutical preparation, development of iopamidol contrast agent comprises several steps, i.e. characterisation of active ingredients, formulation of iopamidol and excipients, analysis of the formulated product, preclinical study and clinical study. To undergo a preclinical study in experimental animals, it is easier to use a radiotracer to study the pharmacokinetics of iopamidol in rats. For this purpose it is suggested to radiolabel iopamidol with Iodine-131, since Iodine-131 ($^{131}\text{I}$) emits gamma rays and this radionuclide is available in our center, CRRT-BATAN. Radiiodination of pharmaceuticals has been previously studied by some researchers as a part of development phase of new drugs. Besides, methods of radiiodination of some chemical compounds have been well established [8-13].

The core of iopamidol chemical structure has similarity with ortho-iodohippurate and MIBG (metaiodobenzylguanidine), and since the protocol of MIBG synthesis is readily established in our center so it was adopted to apply to iopamidol with some modification and optimisation [14-17]. The chemical structures of iopamidol and MIBG can be seen in Figure 1a-1b.

![Fig. 1a. Iopamidol](image1)  ![Fig. 1b. Metaiodobenzylguanidine (MIBG)](image2)

Chemical structure of iopamidol contains some iodine atoms which can be exchanged with radioactive $^{131}\text{I}$ as in the MIBG compound, so the chemical properties will not change. Radiolabeling of some compounds with $^{131}\text{I}$ through isotope exchange scheme has been reported elsewhere, including synthesis of $^{131}\text{I}$ MIBG. Synthesis of $^{131}\text{I}$ MIBG was reported successful through the radiolabeling reaction in acidic at temperature of 160ºC in 60 minutes, resulted final product with radiochemical purity of more than 98% [15-17].

As pure iopamidol is not yet available for this study up to now, commercial product of iopamidol was used under brand name of Iopamiro® (Bracco). Prior to use a preliminary study was carried out, with the aim to ensure that the excipients in Iopamiro product do not interfere in the synthesis of $^{131}\text{I}$ iopamidol.

Since native iopamidol possesses some iodium atoms, the radiolabeling mechanism is assumed to be a cationic exchange through nucleophilic scheme. The iodium atom ($^{127}\text{I}$) is substituted with radioactive iodium ($^{131}\text{I}$) through mechanism of reaction as described in Fig. 2a-2b [18, 19].

![Fig. 2a. Mechanism of reaction through nucleophilic scheme](image3)
2. Material and Methods

Material used are iopamidol contrast agent (Iopamiro®, Bracco), sodium metabisulfite (Merck), copper(II) sulphate (Merck), sodium iodide $^{131}$I (CRRT BATAN), tromethamine (Sigma Aldrich), EDTA and other basic chemicals (Merck).

Synthesis or radiolabeling method was adopted from protocol of MIBG i.e by adding 100 ul of CuSO$_4$ solution (2 mg/ml) to 4 mg of Na$_2$S$_2$O$_3$ in a glass vial, then added with 8 ul of iopamidol and 1 mCi of Na$^{131}$I. The mixture was vortexed, checked for pH and adjusted to pH 3. The vial was capped with a rubber stopper (septum), and inserted with a needle and tube as a ventilator which the other end was punctured into rubber stopper of a vial contained 1 mL of dilute NaOH to trap free iodine (if any). The mixture was heated in a heating block provided with lead shield at 160 °C for 1 hour. The product was reconstituted with 200 ul of water and checked for radiochemical purity using thin layer chromatography [15].

Preliminary study was done by preparing matrix solution consist of EDTA and tromethamine of the same amount as in the commercial product of Iopamiro, then treated with the same protocol to make sure that the excipients in the Iopamiro as a raw material do not interfere in the reaction.
Variation of temperature and reaction time was applied in the experiments to obtain an optimum yield or highest radiochemical purity of $^{131}$I iopamidol. Temperature was varied at 140 °C, 160 °C and 180 °C, while the reaction time was varied from 5, 15, 45 to 60 min.

Analysis of radiochemical purity was carried out using thin layer chromatography (TLC) with TLC-SG as solid phase and mixt of ethyl acetate, glacial acetic acid and water (5:2:1) as eluants. The strips of chromatogram were then analysed with TLC scanner or radiochromatography scanner (Comecer).

Stability study was done using the same methods to see the stability of radiolabeled product during storage at room temperature for several days.

Characterization of both native and radiolabeled iopamidol was also checked using HPLC with C-18 column of 250x4.6 mm and methanol 25% - water as eluants. Flow rate of 1.5 ml/min was applied with gradient flow, and the peak was detected at UV-vis at wave length of 420 nm [20-23].

3. Results and Discussion

Material used for this experiments was iopamidol commercial product (Iopamiro®, Bracco) which contains 30.62 g of iopamidol, 50 mg of tromethamin and 16.5 mg of ethylene diamine tetra acetic acid (EDTA). For preliminary study a matrix solution was made by mixing the same components as in Iopamiro excluding iopamidol. The matrix solution was prepared for radiolabeling with $^{131}$I, and the results showed there was no other peak except the single peak of $^{131}$I (RF 0.0), which means that no reaction occurred between the excipients and $^{131}$I, and concluded that the excipients did not interfere the radiolabeling reaction of iopamidol. (Fig. 3)

![Chromatogram of solution of matrix in the presence of $^{131}$I](image)

**Table 1. Radiochemical purity of $^{131}$I-Iopamidol from various pH**

| pH | Free $^{131}$I as impurity (%) | Radiochemical purity (%) |
|----|-------------------------------|--------------------------|
| 2  | 44.7                          | 55.3                     |
| 3  | 31.2                          | 68.8                     |
| 5  | 28.02                         | 71.98                    |
| 7  | 26.6                          | 73.4                     |
| 9  | 4.69                          | 95.31                    |

3.1. Variation of pH

The protocol of synthesis was adopted from that of $^{131}$I MIBG, i.e the reaction mixture (pH previously adjusted to 3) was heated at 160°C for 1 hour but the result was not good, so the pH was varied from 2 to 9 to obtain radiolabeled product with highest radiochemical purity. The experiment with pH 9 showed the best performance as can be seen in Table 1 and Figure 4 and 5. This pH is suitable for the radioiodinated reaction of iopamidol due to the stability of iodide ion in the solution at this pH.
Variation of reaction time and temperature

Variation of temperature and reaction time resulted in various radiolabeling yield as described in Fig. 6. Experiments using temperature of 140°C and 160°C gave the good results, but the latter required longer time of ~1 hour and unreproducible results (84%±14%) while the experiment using 140°C in 5 min showed reproducible results (96%±1%), so experiment using temperature of 140°C/5 min was concluded as an optimum condition which gave result with higher radiochemical purity of more than 95%.
3.3. Stability study
From the results of experiment the optimum condition has been selected and repeated 3 times to get consistent results, then the product was tested for stability during storage at room temperature for several days. Stability test within 1 week showed the radiolabeled product was still stable up to 7 days of observation. Further observation was not possible since the radioactivity of $^{131}$I has already decreased due to the decay of radionuclide which half-life is 8 days.

3.4. Confirmation of radiolabeled Iopamidol
To prove that iopamidol has been labeled with $^{131}$I, the sample was tested using HPLC provided with UV detector and radioactive inline detector, and native iopamidol was also run for reference. Native iopamidol from certified reference material (CRM, Sigma Aldrich) was eluted at Rt 6.845 min (Fig. 8a), whereas radiolabeled iopamidol showed peaks at Rt 6.875 min and 6.95 min using UV detector and radioactive detector respectively (Fig. 8b and 8c). The close peaks indicated that iopamidol has been successfully labeled with $^{131}$I, and since the peak of radiiodinated iopamidol was close to that of native iopamidol, in other word there was no shift in the retention time, means that the polarity of iopamidol after being radiolabeled did not change. Slight difference in retention time between peaks detected with UV and radioactive detector was due to the distance of both detectors in the HPLC which was passed by the sample during elution.
Fig. 8a. Peak of native iopamidol detected with UV detector, showed at Rt 6.845 min.

Fig. 8b. Peak of radioiodinated iopamidol detected with UV detector showed at Rt 6.875 min.

Fig. 8c. Peak of radioiodinated iopamidol detected with radioactive detector showed at Rt 6.95 min.

**Conclusion**
Iopamidol can be labeled with iodine-131 with high yield and radiochemical purity of more than 90% in at least 5 minutes reaction time with heating at 140°C, and the pH should be adjusted to 9 previously. The radiolabeled product is stable at room temperature up to 7 days, afterwards its radioactivity can no longer be detected due to the decay of the radionuclide.
Acknowledgement

Many thanks to my colleagues in CRRT BATAN who was involved in this research for the participation, and thankful to the Head of CRRT BATAN for the support on this work.

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