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A Simple Preparation of Monoiodobromosulfophthalein-^{131}I by Isotope Exchange for Medical Use

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Abstract

Monoiodobromosulfophthalein-^{131} I (MIBSP-^{131} I), one of the useful radiopharmaceuticals for liver function studies, has been prepared by a simple isotope exchange between the MIBSP and the molecular iodine-^{131} I in phosphate buffer, pH 5.3.

The pooled cold MIBSP was prepared by a normal iodination of BSP using iodine monochloride, and separated from the iodination mixture by applying a Sephadex LH-20 chromatography.

At 100°C, the exchange rate was so fast that the reaction could be terminated in 5 min to show upto 95% yield. The final product could be obtained simply by further heating for about 5 min in a boiling water bath in the presence of a small amount of hydrogen peroxide, and subsequent pH adjustment and membrane filtration.

요 약

간장 질환 진단용 방사성 의약품인 ^{131}I 표지 1 요오드화 브로모프탈레인(MIBSP-^{131}I)을 PH 5.3의 인삼염 완충용액에서 MIBSP와 $^{131}I_2$의 동위원소 교환반응으로 제조할수 있음을 제시하였다.

비방사성 MIBSP는 브로모썰포프탈레인(BSP)을 ICl로 요오드화하고 나서 쎄파덱스 LH-20 크로마토그래피로 분리하여 얻었다. MIBSP와 $^{131}I_2$의 동위원소 교환반응은 매우 빨리 일어나 100°C에서 5분안에 대략 95%의 표지수율을 보였다.

순수한 최종생성물 MIBSP-^{131}I는 미량의 H_2O_2 존재하에 약 5분간 더 반응시킨 후 PH를 조절하고 무균여과함으로써 간단히 얻어졌다.

1. Introduction

Bromosulfophthalein (BSP) has long been used as a diagnosis reagent for the hepatic function as it sharply absorbs 577 mu light when it is contained in the serum in small amount[1]

In terms of radiopharmaceuticals the BSP was firstly labelled with ^{131}I by an ordinary iodination method in 1961.[2] In applying the reported method of ^{131}I labelling, however, it was found that the labelling yield was so sharply dependent upon the reaction conditions that the reproducibility was quite poor. The

usual labelling yield was thus below 20%. Further, the purification process of the repeated precipitation was complicated and the product was still composed of BSP, monoiodo-BSP (MIBSP), and diiodo-BSP (DIBSP) etc. Thus, the method was unsuitable for the routine preparation of the gamma emitting radiopharmaceuticals. Since no alternative method of radioiodine labelling for the compound has been known, the authors applied an iodine isotope exchange method for the MIBSP. Utilizing the MIBSP and $^{131}_{2}I$ an efficient preparation method of the MIBSP-^{131}I has been developed.

2. Experimental

2-1. Preparation of BSP-^{131}I by Direct Radioiodination

BSP-^{131}I was prepared from BSP and $Na^{131}I$ according to the method reported by Manuel Tubis et al[2]. Briefly, to the 1 ml of 0.03 M HCl at 80°C. 0.025 ml (0.5 mmole) of ICl, 5 mCi of $Na^{131}I$ (KAERI) purified by a distillation method[3], 1 ml of 0.26 M BSP (Sigma), and 0.6 ml of 0.1 N NaOH were added in sequence. After maintaining the mixture at 65°C for 2 hrs, it was evaporated to dry. The residue was dissolved into 2 ml of 0.5 N NaOH, poured into 60 ml of cold acetone, and agitated. The resulting precipitate was separated by a centrifugation. Such washing process was repeated three times, and the precipitate was dried in a vacuum desiccator. The labelling yield was determined by radioactivity counting of the dried precipitate using an ionization chamber (Table 1).

2-2. Iodination of BSP

BSP was iodinated according to the method of Manuel Tubis *et al*[2]. However, in present work, the authors used pure non-radioactive ICl instead of radioactive one. Also the molar ratio of the ICl was diminished to about one half of the BSP to get rid of the formation of the diiodo compound. The iodination yield was determined from the weight ratio of the BSP which was separated by a Sephadex LH-20 chromatography (Table 2).

2-3. Separation of MIBSP

A Sephadex LH-20 column chromatography was applied[4]. Small amount of the reaction mixture (composed of BSP, MIBSP, and DIBSP) was applied on a column (45×250 mm) packed 250 g of Sephadex LH-20 (Pharmacia, Sweden). The mixture was eluted with 50% methanol at the rate of 0.2 ml/min. Three

Table 1. Preparation of BSP-^{131}I by direct radioiodination

Run No.	ICl (mmole)	$Na^{131}I$ (mCi)	(ml)	BSP (mmole)	Conditions temp. (°c)	time (hrs)	Lebelling yield (%)
1	0.5	8	1	0.25	65	2	60
2	0.8	5	1	0.30	65	2	12
3	0.5	5	1	0.30	65	2	15
4	0.5	5	1	0.30	80	2	5
5	1.0	3	1	0.25	65	2	5
6	—*	3	0.5	0.25	65	2	5

* 2 mg chloramine-T was used

separated blue bands were separately collected, dried and weighed. The component of the each fraction was identified by TLC, UV-, and NMR-spectroscopy (Fig. 1 & Fig. 4)

2-4. Isotope Exchange Reaction

Iodine isotope exchange reaction between MIBSP and $^{131}I_2$ was conducted in 0.5 M phosphate buffer, pH 5.3. The reaction conditions were just similar to those for the preparation of Rose Bengal-^{131}I[5].

$$\text{MIBSP}-{}^{127}\text{I}+{}^{131}\text{I}_2 \underset{\text{p. buffer, 100°C}}{\rightleftharpoons} \text{MIBSP}-{}^{131}\text{I} + {}^{127}\text{I}_2 \quad \cdots\cdots (1)$$

Table 2. Iodination of BSP using iodinemono chloride

Run No.	ICl (mmole)	BSP (mmole)	Conditions temp. (°C)	Conditions time (hrs)	Iodination yield (%)
1	0.5	0.25	65	2	82
2	0.5	0.25	65	3	86
3	0.5	0.20	65	2	77

Thirty mg of MIBSP was dissolved to the phosphate buffer. To this solution about 5 mCi of $Na^{131}I$, pH 8-9, containing reducing agent (KAERI) and 0.1 ml of 30% H_2O_2 were added. The whole content was tightly closed and heated in a boiling water bath for 5 min or more. The variations of the exchange rates with pH, salt content, and reducing agent were measured (Fig. 2 & Fig. 3). In the measurement of the rates, a radio-paper chromatography technique using n-BuOH: H_2O: HAc 1. 48:1:0.37 (v/v) [4] was applied (Fig. 4).

2-5. Purification of the Exchange Product

To get the pure MIBSP-^{131}I, the reaction mixture was heated further for 5 min in the presence of further 100 ul of 30% H_2O_2 without closure of the reaction tube. By this treatment, the labelling yield exceeded 95 %. The TLC data showed the radiochemical purity more than 95%.

3. Results and Discussion

The average labelling yield obtained by the direct rodioiodination was about 20% (Table 1). The labelling yield of about 60% was once obtained but the reaction conditions were so sensitive that such yield could no more be obtained.

However, the average yield of the non-radioactive iodination was high enough to show 80% (Table 2). Since the reaction conditions were same as those for the direct radioiodination, the cause of the low and the non-reproducible yield in the direct radioiodination would be attributable to the presence of some impurities such as sodium thiosulfate, sodium chloride, and trace amount of the target material etc. in the sodium radioiodide solution.

In the separation of the MIBSP, the BSP was eluted first, then the MIBSP, and finally the DIBSP. Considering the separation pattern correlating with the molecular weights and structures, the chromatography implied not the mechanism of the usual Sephadex filtration but predominantly those of the partition and the adsorption. Generally, the sepation efficiency was high enough even though it took more than 20 hrs to complete the separation: The maximum absorption wave lengths for the BSP, the MIBSP, and the DIBSP were 577, 689, and 600 mu, respectively, which were coincided well with those in the literature [4]. The NMR spectra indicated that the iodine substitution occured at the ortho position (s) of the phenol ring in the BSP (Fig. 1). For the DIBSP, a single peak at 7.8 ppm was originated from the total of four hydrogen atoms at the non adjacent positions (2′, 6′, 2″, and 6). For the MIBSP, a relatively

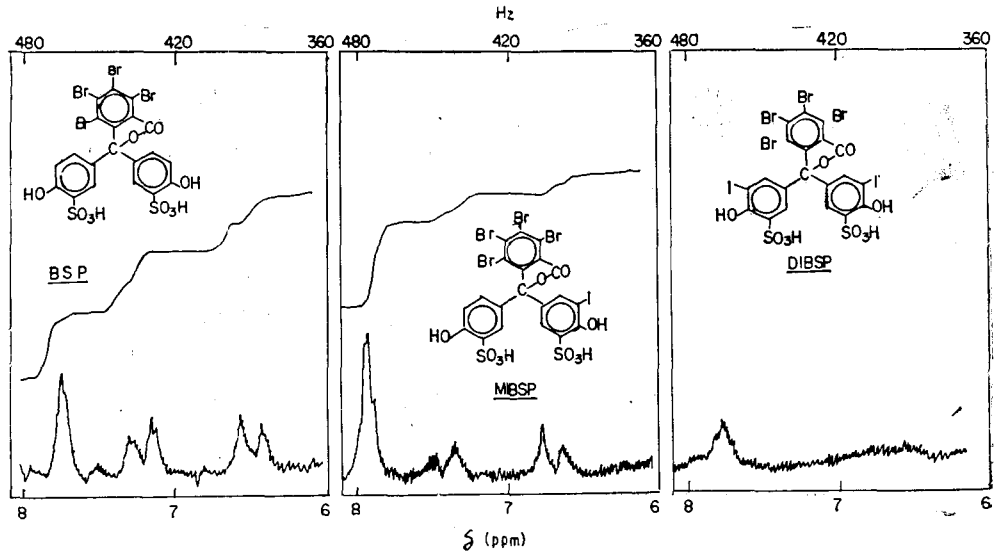

Fig. 1. N.M.R. Spectra

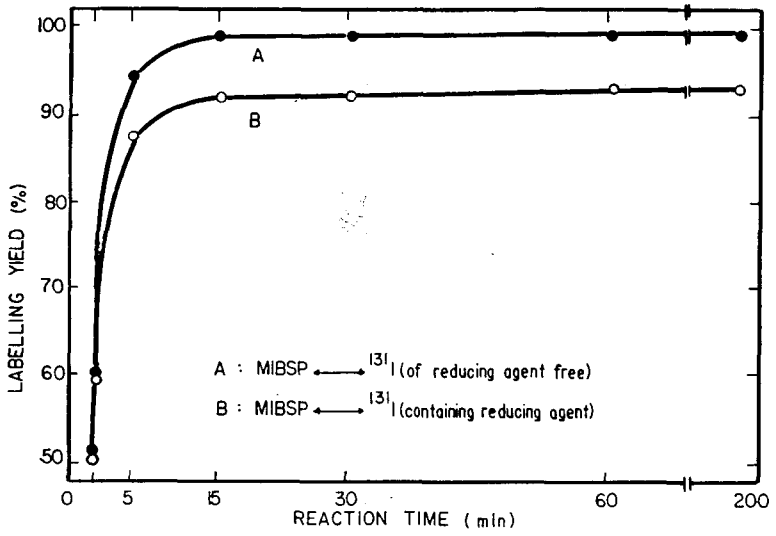

Fig. 2. The exchange rates in the phosphate buffer, pH 5.3

stnong peak at 7.9 ppm was originated from the two hydrogens of the non adjacent positions (6′ and 6″). The peak of the 2″ hydrogen was splitted into two by the adjacent 3″ hydrogen and the other C-H peak originating from 3″ hydrogen was also splitted into two by the 2″ hydrogen. Likewise, the BSP showed a decoupled peak originating from 6′, 6″ hydrogens, and two splitted peaks originating from the 2′, 2″ hydrogens and 3′, 3″ hydrogens. In the TLC uring the SiO_2 gel plate (Kodak 6061) and the solvent system of n-BuOH:H_2O:HAc 1.48:1:0.37 (v/v), each compound showed distinct Rf values (Fig. 4). Thus each component of the eluate was identified.

For conducting the isotope exchange, the MIBSP was, at first, obtained by the non-radioactive iodination of the BSP, then separated clearly from the reaction mixture

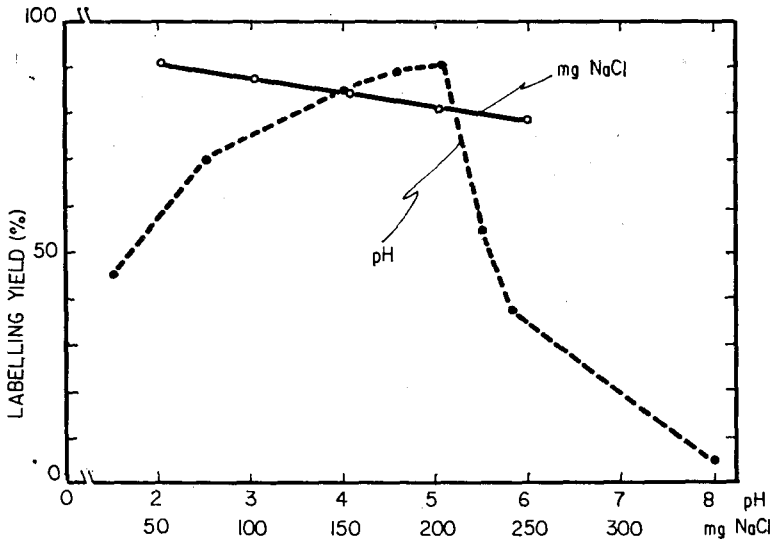

Fig. 3. Dependence of the exchange labelling yield upon the pH and NaCl content

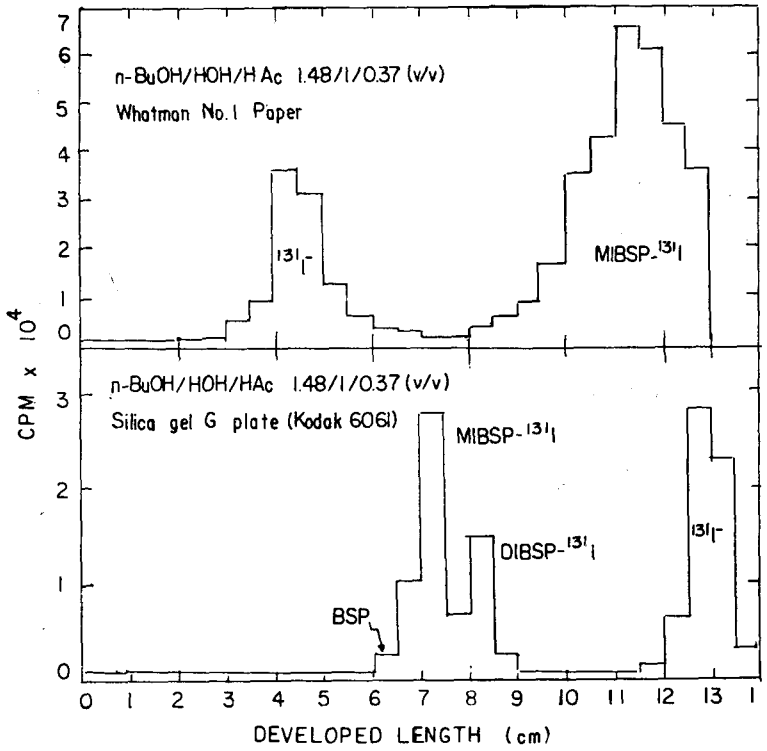

Fig. 4. Paper-and thin layer-chromatograms

by the chromatography, and pooled in bulk.

In the isotope exchange, the pH 5.3 was the optimum, the less NaCl content showed the better yield, and the presence of the reducing agent degenerated the yield (Fig. 2 & Fig. 3).

The exchange yield was, however, not much influenced by the presence of the reducing agent, and the reaction condions could be maintained steady to reveal the good reproducibility. The reaction proceeded smoothly without any side reactions. Thus, it was quite suitable for the routine preparation of the pure MIBSP-^{131}I. Previously, one of the authors reported that the 5-bromouracil-^{82}Br could be prepared by the bromine isotope exchange between 6-bromouracil and $^{82}Br_2$ in aqueous solution[6], and the p-aminosalicylic acid (PAS)-^{131}I was also prepared by iodine isotope exchange[7]. The Br or I atoms in aromatic ring could be easily exchanged under a definite condition with radioactive isotopes especially when the reaction site was activated by some substituents.

In case of the direct radioiodination of BSP according to the known method[1], the purification method was complicated, and even though the purified product was free from the unlabelled radioiodine the product was still composed of the BSP, the MIBSP and the DIBSP.

It has been known that the blood clearence rate of the MIBSP is faster than that of the DIBSP[4], and thus the monoiodo compound is superior to the diiodo compound in the radiopharmaceuticals point of view. In this respect, the isotope exchange method is decisively more suitable than the direct radioiodination method.

4. Conclusion

As the iodine isotope exchange between the MIBSP and the $^{131}I_2$ is quite fast, it can be applied for the routine preparation of the MIBSP-^{131}I. The exchange yield is about 95% in 5 min at 100°C. The MIBSP-^{131}I of more than 95% radiochemical purity can be obtained by further 5 minutes reaction in the presence of a small amount of 30% H_2O_2 The specific radioactivity of the product is more than 160 uCi/ug.

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