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Study on Iodine Labelling (II)

Efficient Methods of Labelling Rose Bengal, Hippuran, and Human Serum Albumin in Small Scale

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Abstract

For efficient micro scale syntheses of Rose Bengal-^{131}I, Hippuran-^{131}I, and H. S. A.-^{131}I, the dependence of labelling yields on pH, on salt contents, and on the volume of buffer solution in the reaction mixtures as well as the reaction apparatus were studied.

pH of 5.6 was optimum for preparation of both Rose Bengal-^{131}I and Hippuran-^{131}I but pH of 8.5 was optimum for preparation of H. S. A.-^{131}I. Salt in the reaction mixtures hindered drastically the formation of Hippuran-^{131}I but it slightly increased the labelling yield of H. S. A.. The compactly closed reaction vessels were effective for preparations of both Rose Bengal-^{131}I and Hippuran-^{131}I in small volume.

Thereupon, the labelling procedures were modified to bring about higher labelling yields and better reproducibilities.. By these newly established procedures, the labelling yields of Rose Bengal-^{131}I and Hippuran-^{131}I could be increased even with the home-produced sodium iodide-^{131}I solution containing reducing agent.

요 약

Rose Bengal-^{131}I, Hippuran-^{131}I, H. S. A-^{131}I 등을 효과적으로 합성하기 위해 표지반응액의 pH, 염의함량, 반응액중의 완충용액의 부피 및 합성장치등에 따르는 표지반응수율을 검토하였다.

Rose Bengal-^{131}I 및 Hippuran-^{131}I의 반응수율은 pH 5.6에서, H. S. A-^{131}I의 반응수율은 pH 8.5에서 각각 가장 좋았다.

반응액중에 함유된 염은 Hippuran-^{131}I의 생성반응을 크게 저해 시켰으며 H. S. A.의 표지수율은 어느 범위안에서 오히려 약간 향상시켰다.

Rose Bengal-^{131}I 나 Hippuran-^{131}I를 소규모 합성할 경우는 밀폐된 용기가 효과적이었다.

이상의 결과에 따라 더 높은 표지수율과 좋은 재현성을 얻을 수 있는 반응조건을 확립하였으며 이에 따라 환원제가 함유된 국산 Na^{131}I를 사용하드래도 Rose Bengal-^{131}I과 Hippuran-^{131}I의 표지수율을 높일수 있었다.

1. Introduction

For efficient micro scale syntheses of Rose Bengal-^{131}I, Hippuran-^{131}I, and H. S. A.-^{131}I, the establishment of optimum reaction conditions is very important. According to the previous report[1], the labelling yields of Rose Bengal-^{131}I, Hippuran-^{131}I and H. S. A.-^{131}I are 80%, 70% and 80%, respectively, by using home-produced sodium iodide-^{131}I solution of reducing agend-free and of low iodate concentration. However, the reproducibilities of labelling yields under the conditions previously established are so poor that we have often encountered much difficulties in routine syntheses. The cause of the poor reproducibilities may be attributable to the inadequate pH of the reaction mixture and the variation of sodium chloride contents in the reaction mixtures. The contents of sodium chloride in sodium iodide-^{131}I solution may be varied with the used amounts of sodium hydroxide and hydrochloric acid to control the pH of the solution either in the production process of sodium iodide-^{131}I from reactor, or in the process of further purification[1]. To make higher specific radioactivity in mCi/ml., the sodium radioiodide solution should be concentrated prior to use it for labelling. Consequently, the concentration of sodium chloride can not be negligible especially for the radioiodide solution of which pH was adjusted twice or more by using large amounts of acid and base. It is well known that the electrolytes such as sodium chloride, sodium carbonate or thiosulfate in the reaction mixture will certainly influence on the reaction rates owing to the increased ionic strength[2].

To prevent the probable evaporation of radioiodine and consequent loss of radioactivity, it is further desirable to conduct the reactions in closed vessels especially for those being conducted in acidic medium. In general, simple and prompt labelling methods are valuable in view of radiation protection, radioactive decay and decompositions of labelled molecules due to the various radiation effects.

In this paper, the author has investigated the yield dependence on pH, on the contents of sodium chloride and on the volume of buffer solution in the reaction mixtures as well as some modifications of reaction apparatus to enhance the labelling yields and to bring about better reproducibilities of the labelling yields in small scale routine syntheses.

2. Experimental

(1) Materials

Three kinds of sodium iodide-^{131}I solution were procured and confirmed their purities and characteristics;

a) Sodium iodide-^{131}I containing reducing agent (6mM $Na_2S_2O_3$), (abbrev. $Na^{131}I(A)$): it was provided by radioisotope production group, K. A. E. R. I. The pH of the solution was around 8, and its specific activity was about 1mCi/ml. It was condensed by evaporation to meet 4—5 mCi/ml. The condensed solution was subjected to qualitative test for confirming the presence of sodium chloride. In some condensate visualy detectable turbidity was formed when 0.1 N silver nitrate solution was dropwisely added to 1ml. of the sodium iodide-^{131}I solution. Iodate-$^{131}I^-$ or periodate-$^{131}I^-$ could not be detected by radio paper chromatographical separation and subsequent gamma-counting of the chromatostrips. Whatman No. 1 filter paper, and 75% methanol were used in the chromatography.

b) Sodium iodide-^{131}I solution of reducing agent-free, (abbrev. $Na^{131}I(B)$): the reducing agent in $Na^{131}I(A)$ was removed by distillation

in accordance with the previously reported method[1]. pH of the distillate was adjusted to 8 with sodium hydroxide solution and hydrochloric acid. It was condensed to meet 3—4 mCi/ml. of specific activity. Less than 1% of iodate-^{131}I was detected. The presence of sodium chloride could be confirmed as for Na^{131}I(A) only for the condensate of which pH was adjusted with considerable amounts of acid and base.

c) Sodium iodide-^{131}I solution of reducing agent-free and salt-free, (abbrev. Na^{131}I(C)); Na^{131}I(A) was distilled using sulfuric acid and hydrogen peroxide in the same way as reported previously[1] but in this case, 15ml. of very dilute solution of sodium hydroxide (pH=8.0), made by dropwise addition of 0.01 N sodium hydroxide to large amount of distilled water using pH meter, was applied as trapping solution for the distillate of radioiodine. The distillation was carried out keeping mild conditions so as to prevent iodate-^{131}I formation. The recovery % was disregared since the only objective was the preparation of sodium iodide-^{131}I free from salt, reducing agent, and iodate-^{131}I. The pH of the distillate was nearly constant. It was condensed to 1-2 mCi/ml. of specific activity. Less than 1% of iodate-^{131}I$^-$ was detected. No turbidity occured when it was treated with silver nitrate solution.

(2) Experimental Procedure

a) Labelling Yield Dependence on pH.

(i) Rose Bengal-^{131}I Preparation; Acetate buffer solution of different pH were made by varying mixing ratios of 1M sodium acetate and 1M acetic acid. In each small reaction tube 2 ml. of the actate buffer, 25 mg. of pure Rose Bengal(tetrachloro tetraiodofluorescein). 2mCi of Na^{131}I(B) (less than 0.5 ml.) and 1 drop of 30% hydrogen peroxide. The reaction mixture was heated for 3 hrs. on water bath

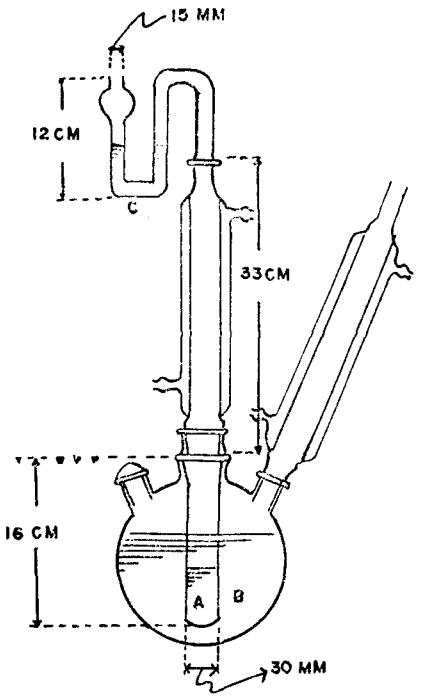


Fig. 1. The preparation apparatus for Rose Bengal-^{131}I or Hippuran-^{131}I A; reaction vessel, B; water-bath, C;NaOH-$Na_2S_2O_3$ trapping solution.

using the apparatus shown in Fig. 1.

Separations of the unbound ^{131}I from Rose Bengal-^{131}I were achieved by radio paper chromatography using Whatman No. 1 filter paper and 0.25 M sodium citrate. The radioactivity on the separated chromatostrips was counted by using conventinal scintillation counter. Since, conceivable radioactivities resulting from the decomposed products or isomers of Rose Bengal could not be found, the labelling yieles were calculated accoding to the following equation;

$$\text{labelling yield}(\%) = \frac{\text{CPM(from Rose Bengal—}^{131}\text{I)}}{\text{CPM(from Rose Bengal—}^{131}\text{I)} + \text{CPM(from unbound}^{131}\text{I)}} \times 100 \quad (1)$$

Upon the measurement of the radioactivity in the trapping solution (15—20 ml. of 1 M NaOH/1 M $Na_2S_2O_3$=1/1(V/V) settled on the top of the water-cooled condenser(Fig. 1)), it

was found that about 7% of the originally applied activity was trapped.

(ii) Hippuran-^{131}I Preparation; the reaction conditions were the same as that of Rose Bengal—^{131}I preparation except that the added amounts of pure Hippuran (sodium o-iodohippurate) was 300 mg. and the reaction time was 7 hrs. Separation of Hippuran-^{131}I and unbound ^{131}I was achieved by paper chromatography. Whatman No. 1 filter paper and the solvent system of n-butanol/acetic acid/water (4/1/1, V/V) were used. The activities on the chromatostrips were counted. Since there were only two activity-peaks resulting from the labelled Hippuran and unbound^{131}I the labelling yields were determined by applying equation (1). Upon the measurements of the radioactivities in the trapping solution afore-described, it was found that average 10% of the originally used activity was trapped.

(iii) H. S. A. -^{131}I Preparation; a series of radioiodination on human serum albumin in different pH of phosphate buffer was carried out; 100 mg. of normal human serum albumin was added to 3 to 4 ml. of phosphate buffer. To this solution 2 mCi (less than 0.5 ml.) of Na^{131}I (B) and 1 ml. of chloramine-T solution (400μg./ml) were added with stirring. After 30 min. at 30°C, 0.5 M sodium sulfite solution was added. The labelling yields were determined in the same way as that of Rose Bengal-^{131}I but, in this case, 75% methanol was used as developing solvent in the paper chromatography.

b) Labelling Yield Dependence on Salt Contents.

Labelling experiments of Rose Bengal, Hippuran and H. S. A. were, respectively carried out in the same way as that of Section a) but using Na^{131}I(C), and adding different amouts of sodium chloride in the reaction mixtures. The pH of the reaction mixtures of both Rese Bengal-^{131}I and Hippuran-^{131}I was 5.6 and that for H. S. A. -^{131}I was 8.5 which were the optimum pH resulted from the experimental section a).

c) Labelling Yield Dependence on the Volume of Buffer Solution in the Reaction Mixture

The preparation experiments for the three labelled compounds were carried out in similar way to that of section a) but adding different amounts of buffer solution into the raction mixture.

According to the results obtained in the experimental sections of a) and b), the acetate buffer of optimum pH 5.6 and Na^{131}I(C) were used for labelling Rose Bengal and Hippuran, and the phosphate buffer of pH 8.5 and Na ^{131}I(B) of salt-rich were used for labelling H. S. A.

d) Labelling Rose Bengal, Hippuran and H. S. A. under Optimum Conditions.

According to the results obtained from preceding experiments, the labelling conditions for the three labelled compounds were newly established and two series of experiments were carried out in accordance with the newly established procedures using Na^{131}I(A), and Na^{131}I(B);

(i) Rose Bengal-^{131}I; into an open Pyrex ampoule of about 10 ml. capacity 2 ml. of acetate buffer (pH 5.6), 25 g. of pure Rose Bengal, less than 0.5 ml. of salt-poor Na^{131}I (B) or salt-poor Na^{131}I(A) and 1 drop of 30% hydrogen peroxide solution were added, respectively. The distinction of salt-poor Na^{131}I (A) or Na^{131}I(B) from the salt-rich Na^{131}I(A) or Na^{131}I(B) could be carried out by visual detection of the degree of turbidity formation when 0.1 N silver nitrate solution was added to a fraction of the definitely condensed (*eg.*, 25 ml. to 2 ml., 50 ml. to 4 ml.) Na^{131}I(A) or Na^{131}I(B) solution. The ampoule was imme-

diately encapsulated and subsequently heated on water-bath for 3 hrs. The ampoule was ice-cooled and opened. The labelling yields were determined in the same way as a), (i).

(ii) Hippuran-^{131}I; the procedure was the same as that of Rose Bengal-^{131}I except that the added amounts of Hippuran was 300 mg., acetate buffer (pH 5.6) was 1.5 ml. and the reaction time was 7 hrs. The labelling yields were determined in the same way as a), (ii).

(iii) H. S. A. -^{131}I; to 2.5 ml. of phosphate buffer(pH 8.5) 100 mg. of normal H. S. A. was dissolved. To the solution less than 0.5 ml. of Na^{131}I(B) (preferably salt-rich) and 1.5 ml. of freshly prepared chloramine-T (0.4 mg. /ml.) solution were added. The reaction mixture was shaken and set aside at 30°C for 1 hr. Then 0.5 ml. of 0.5 M sodium sulfite solution was added. The labelling yields were determined in the same way as that of a), (iii) (Table 1).

3. Results and Discussion

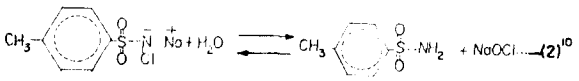
(1) Labelling Yield Dependence on pH

As Fig. 2 shows, the labelling yield of Rose Bengal-^{131}I is gradually incrased with pH and eventually reaches maximum at pH 5.6. Above this pH value, the yield decreases sharply.

This data indicate that the reaction mixture should be kept the pH of below 5.6. Mani, R. S. reported that the pH of the the Rose Bengal-^{131}I reaction mixture should be below 6[3] and is optimum at pH 5.0[4]. In view of safety, the data obtained by Mani, R. S., and P. Raban and Gregora[5] may be correct. However, in present study the optimum value was found to be 5.6 rather than 5.0. In the small scale synthess, keeping the exact optim um pH is further desirable comparing the larger scale practice.

pH of 5.6 is also optimum for Hippuran-^{131}I preparation. This value is slightly different from the data obtained by N. Kaltenborn (pH 6)[6], Walter Harrodt (pH 5.7-6.0)[7], Mani. R. S. (pH 5)[3], and C. J. Anghileri (pH 4.5)[8] but much different from that of K. E. Scheer and W. Meiser-Brost (pH 1.8)[9]. It has been confirmed that the pH of 1.5 ml. of acetate buffer is sufficiently maintained its own pH in case of being added less than 0.5 ml. of Na^{131}I(A) or Na^{131}I(B) solution. Therefore, the stock solution of acetate buffer (pH 5.6) may be conveniently used for the preparation of both Rose Bengal-^{131}I and Hippuran-^{131}I. The optimum pH for prearation of H. S. A. -^{131}I is 8.5. This value is slightly higher than the previously known (pH 7.2—7.4)[3,6]. It is confirmed that the 2 to 4 ml. of phosphate buffer sufficiently maintaines its own pH in case of being added 1 ml. of chloramine-T solution (400 Mg. /ml., pH 7.6) and less than 0.5 ml. of Na^{131}I(B) solution.

As far as the electrophilic substitution mechanism is involved in this reaction, any one step of the following reaction sequence seems to be catalyzed by general or specific base (pH 8.5).



$$NaOCl \rightleftharpoons NaO^- + Cl^+ \quad (3)$$

$$Cl^+ + {}^{131}I^- \longrightarrow Cl^- + {}^{131}I^+ \quad (4)$$

It is expected that Cl^+ will react with $^{131}I^-$ to form $^{131}I^+$, which is the electrophile involved in the electrophilic substitution at tyrosine ring[11]

(2) Labelling Yield Dependence on Salt Contents

As shown in Fig. 3, increased salt contents degenerate the labelling yields in the preparation of Rose Bengal-^{131}I and Hippuran-I. It is considered that the variation of yields with the salt contents in small scale reaction mixture is especially fatal. The labelling yield is sharply decreased with increasing sodium

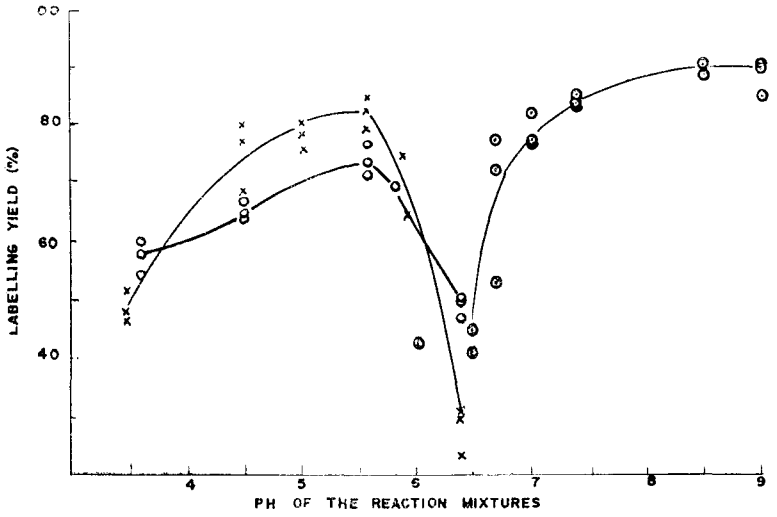


Fig. 2. Dependence of labelling yield on pH of the reaction mixture.
-○- : Hippuran-^{131}I, -×- : Rose Bengal-^{131}I, -⊙- : H. S. A-^{131}I

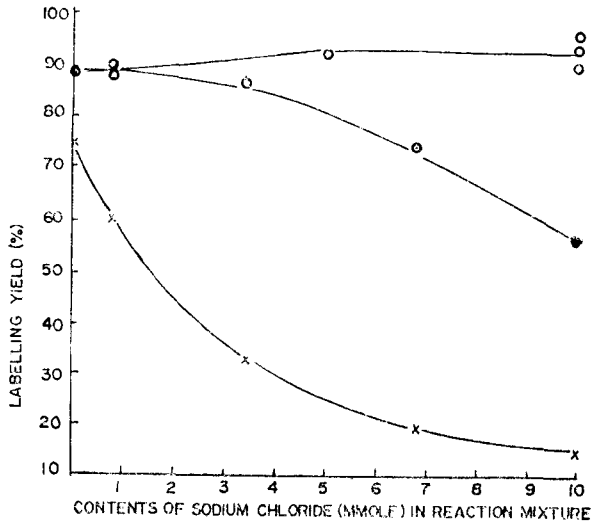


Fig. 3. Dependence of labelling yield on the contents of sodium chloride in reaction mixture.
-⊙-: Rose Bengal-^{131}I, -×-: Hippuran-^{131}I, -○-: H. S. A-^{131}I

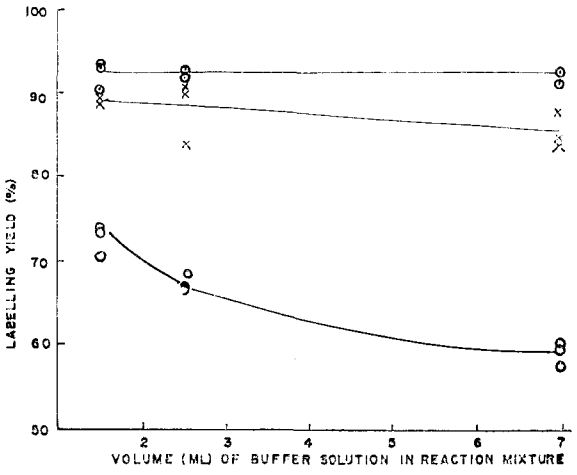


Fig. 4. Dependence of labelling yield on the volume of buffer solution in the reaction mixture.
-○-: Hippuran-^{131}I, -×-: Rose Bengal-^{131}I, -⊙-: H. S. A-^{131}I

chloride contents in the preparation of Hippuran-^{131}I. Due to the facile exchange the dependence of labelling yield on salt contents in Rose Bengal-^{131}I preparation is almost negligible when less than 3 mmole of sodium chloride is contained in 2.5 ml. of reaction mixture. The exchange labelling yields of Rose Bengal-^{131}I are generally fair.

In the radioiodination of H. S. A., however, the labelling yield is slightly proportional to the sodium chloride contents in range of 1 to 8.5 mmole in 4.5 ml. of reaction mixture. However, the slight proportionality of labelling yield on salt contents is hardly explained and still in doubt. Anyway, the result strongly suggests that Na^{131}I(B) can be used for labelling

H. S. A. regardless the salt contents in $Na^{131}I$ (B).

(3) Labelling Yield Dependence on Volume of Buffer Solution In the Reaction Mixture

As Fig. 4 shows, the labelling yield is decreased with increasing volume of acetate buffer in the Hippuran-^{131}I preparations, but the volume change of buffer solution is only a trifling factor for preparation of Rose Bengal-^{131}I. The obtained data indicate that the volume of the buffer solution should be minimized keeping higher concentrations of reactants especially in preparation of Hippuran-^{131}I. Such a trend is coincide with that in the literature;[12] in the isotopic exchange reaction between Hippuran and potassium iodide-^{131}I follows first order kinetics to Hippuran.

It is also expected that the closed reaction vessels for those being conducted in acidic medium will be better than the open ones, especially in small scale practice. In acidic medium of pH 5.6, radioiodine will escape from the reaction mixture in the chemical form of either $H^{131}I$ or $^{131}I_2$ which are formed by oxidation. The results of the measurements of radioactivity trapped in the alkaline-thiosulfate trapping solution settled upon the top of the water-cooled condenser (Fig. 1) indicated that much radioactivity had been trapped; *ie*, in case of Rose Bengal-^{131}I preparation it was average 7%, and in case of Hippuran-^{131}I preparation it was average 10% of the total activity applied, respectively.

Considering the obtained data so far, the preparation methods for the three labelled compounds should be modified; (1) the pH of the reaction mixture should be 5.6 for preparation of both Rose Bengal-^{131}I and Hippuran-^{131}I, and should be 8.5 for preparation of H. S. A.-^{131}I, (2) $Na^{131}I$(B) of preferably salt rich is suitable for preparation of H. S. A.-^{131}I or even of Rose Bengal-^{131}I but not for preparation of Hippuran-^{131}I, (3) the exchange reactions being conducted in acidic medium and in small volume should be carried out in closed vessels, and the volume of reaction mixture should be minimized especially for Hippuran-^{131}I preparation.

(4) Labelling Rose Bengal, Hippuran and H. S. A. under Optimum Conditions

As Table 1 shows, the average labelling yields under modified conditions are higher than those of the previously obtained. The labelling yields (Y_1 in Table 1) are decreased 5-10% for Rose Bengal-^{131}I, and 10-20% for Hippuran-^{131}I, respectively, in case of using $Na^{131}I$(A) even though the reactions were followed by newly established procedures. However, they are still higher than that obtained via previously established procedures. On the other hand, the influence of reducing agent in sodium iodide-^{131}I solution on labelling H. S. A. is still drastic and it is almost imposssible to label H. S. A. even though the reactions were followed by the newly established procedure. It is attributable to the insufficient formation of electrophile, $^{131}I^+$, owing to the presence of reducing agent.

Table 1. Comparison of labelling yields

Compounds	Established Procedures	Labelling Yields(%)	
		Y_1	Y_2
Rose Bengal-^{131}I	previous	30—35	75—80
	new	60—80	85—90
Hippuran-^{131}I	previous	15—20	60—70
	new	50—55	70—75
H. S. A.-^{131}I	previous	5(max.)	75—80
	new	8(max.)	90—97

Y_1; yields obtained by using $Na^{131}I$(A), determined by application of equation (1)

Y_2; yields obtained by using $Na^{131}I$(B), determined by application of equation (1)

Even though the $Na^{131}I$(C) is the most suitable for Hippuran-^{131}I preparation it is very difficult to be made routinely due to the unavoidable significant activity loss in the purification. The increased labelling yields (Y_1) in the Rose Bengal-^{131}I and Hippuran-^{131}I preparations are, therefore, attributable mainly to the optimum pH, closed reaction vessels, and salt-poor $Na^{131}I$. In practical bases, the distinction of salt-poor $Na^{131}I$ from salt-rich $Na^{131}I$ is quite valuable only in case of Hippuran-^{131}I preparation. Quantitative measurements of sodium chloride contents can be accomplished very simply in case of using commercialy available sodium chloride concentration indicator.

Anyway, in present status, the troublesome and time-consuming work for removing reducing agent from $Na^{131}I$(A) is unnecessary as far as the preparations of Rose Bengal-^{131}I and Hippuran-^{131}I are concerned.

In the preparation of H. S. A. -^{131}I, the yields are increased in average 10% more than that obtained previously (Y_2 in Table 1). In newly established procedure, the pH, the amounts of buffer solution, and the reaction time are adjusted. Since, the criterion of radiochemical purity in U. S. P. is 95%[13], the products of H. S. A. -^{131}I (in case of labelling yield is in range of 95-97%) can be used without further purification. The other important factor influencing on labelling yield of H. S. A. is the freshness of the chloramine-T solution[14].

4. Conclusion

The preparation methods of Rose Bengal-^{131}I, Hippuran-^{131}I, and H. S. A. -^{131}I are modified to bring about better reproducibilities and higher labelling yields. In accordance with the newly established procedures, the preparations of Rose Bengal-^{131}I and Hippuran -^{131}I can be accomplished even by using home -produced sodium iodide-^{131}I solution containing reducing agent but salt-poor, without any difficulties in small scale routine work.

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