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Labelling of Some Iodinated Organic Compounds by Halogen Exchange in Organic Media

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ABSTRACT. The optimum Parameters of labelling rose bengal in a polar organic medium are discussed. The halide exchange with inactive rose bengal gave over 90% yield within one hour reaction time with undegraded final product. This generalised method can be applied successfully to label any iodinated organic compound, such as hypuran, thyroxine, tyrosine or aliphatic fatty acids, for application in nuclear medicine.

The great potential of I-123 labelled compounds and radiopharmaceuticals (Stocklin 1977) necessitates basic research and development of efficient, rapid and mild labelling procedures with emphasis on the preparation of practically carrier free products. In principle, halogen exchange is an important method of introducing radioiodine in the molecule. The exchange can be isotopic and nonisotopic, either electrophilic *via* positive halogen or nucleophilic *via* halide ions, or even a homolytic process *via* halogen atoms. It can proceed in solution or in molten organic systems (Elias *et al.* 1973).

In this study, we used the isotopic exchange technique with inactive iodine as carrier for radioiodine. With no carrier the labelling yield is only 45%. The exchange is conducted in a polar organic solvent at its boiling point. Through homolysis the I_2 molecule furnishes a positive iodine atom. As an illustration of this generalised technique, we studied the labelling of iodinated rose bengal by halogen exchange.

Rose bengal labelled with radioiodine is commonly used for liver function studies (Silver 1968). It was usually prepared by exchange reaction between inac-

tive rose bengal and $Na^{131}I$ in aqueous acetate buffer at 80°C for 4 to 8 hr with a yield of 80% and some degradation products (Liebster and Andrysek 1959, Hallaba and Raieh 1966). Raban and Gregora (1968) described a new method of exchange in a boiling organic solvent to obviate the degradation problem with an 80-85% yield within 3 to 4 hr. Labelling rose bengal in acid or alkaline medium may produce lower halogenated components, but this is minimized in neutral medium.

In this investigation, we optimised and improved Raban's conditions by control of the different factors affecting the mechanism of the exchange reaction. This was achieved by using different organic media, different concentrations of carrier I_2, different reaction temperatures and adjusting the polar solvent to different pHs where different forms of rose bengal may prevail such as the lactone form or the mono or the disodium salt (Hallaba *et al.* 1981)

Experimental

As inactive rose bengal we used the certified Aldrich chemical (Aldrich Rose Bengal certified by the Biological Stain Commission Inc., M.W. 1017.65 dye content 92%). It proved to be a high quality material. Nevertheless, we purified it on sephadex G-25 (Desai and Hegesippe 1968) and found that no difference existed between the purified collected fractions and the original certified chemical. We used directly the certified R.B. without any further purification. As radioiodine, we used iodine-125 (Amersham product) for its longer shelf life and low radiation hazards. Paper chromatography on Whatman No 3 paper was used to determine the degree of labelling using the ascending technique with a developing solvent of ethanol:ammonia:water in the ratio (10:8:82) or *n*-butanol:acetic acid:water (4:1:1). A Beckmann gamma counter 5500 was used to determine the activity of the paper strips of the chromatogram.

General Labelling Technique

The labelling procedure applied in this study comprises three steps:

A) In a small double neck 100 ml round bottom flask 10 mg of the pure R.B. (10 μmole) are dissolved in 10 ml of 96% doubly distilled ethanol.

B) 2 μmole of inactive iodine incorporating the radioiodine I-125 in 3 ml organic solvent are prepared in a well ventillated lead shielded fume hood in the following way:

In a well stoppered separatory funnel, 1.5 ml KIO_3 solution (2 mg/ml), 0.5 ml KI solution (1 mg/ml), 0.1 ml carrier free $Na^{125}I$ (one μCi)*, 3 ml organic solvent and 0.1 ml of 1 N H_2SO_4 were gradually added. After gentle shaking for 5 min, the organic layer is carefully separated and added to solution A.

C) A double surface condenser is fixed in one neck of the flask while the other is blocked by a glass stopper. The mixture was gently refluxed. The labelling is followed by withdrawing from time to time 0.1 ml of the mixture, adding it to 0.1 ml thiosulphate solution (1 mg/ml) to fix the free iodine as iodide and analyzing this solution chromatographically.

Results and Discussion

Isotopic exchange methods require a certain activation energy which may be obtained by heat (thermal agitation), light (ultra-violet) or ionising radiations.

If one compares the bond energies (Moore 1965), one may reasonably conclude that exchange reaction of the type

$$R - C - X + X^* \leftrightharpoons R - C - X^* + X$$

will take place most easily at the highest rate if X is an iodine atom as the bond energy is the lowest for the C–I pair (56 kcal/mole).

Table 1. Single bond energies for C—X bonds (kcal/mole)

X	=	F	O	N	Cl	Br	I	C
C	=	102	81	62	77	64	56	80

A) *Effect of Temperature*

The above equilibrium exchange is shifted to the right as the temperature is increased. The C—I bond in the organic compound excited by heat energy is weakened and the free radioiodine atom can exchange easily with the inactive iodine-127 in the compound. Such results are seen in Fig. 1 where labelling at 30°C is lower than at the boiling point of the solvent and the rate of exchange is slower.

B) *Effect of Carrier*

A minimum concentration of inactive iodine is necessary to reabsorb the liberated halide ion due to homolytic effect until all the radioiodine is consumed

$$I_2 + OH^- \rightarrow OHI^* + I^-$$

in the exchange reaction since over 90% yield is obtained and the splitting of only I_2 would give a 50% yield. This is quite clear from Fig. 2 where a concentration of 0.2 μmole of I_2 per 1 μmole of rose bengal is optimum for the maximum yield

* μCi = microcurie.

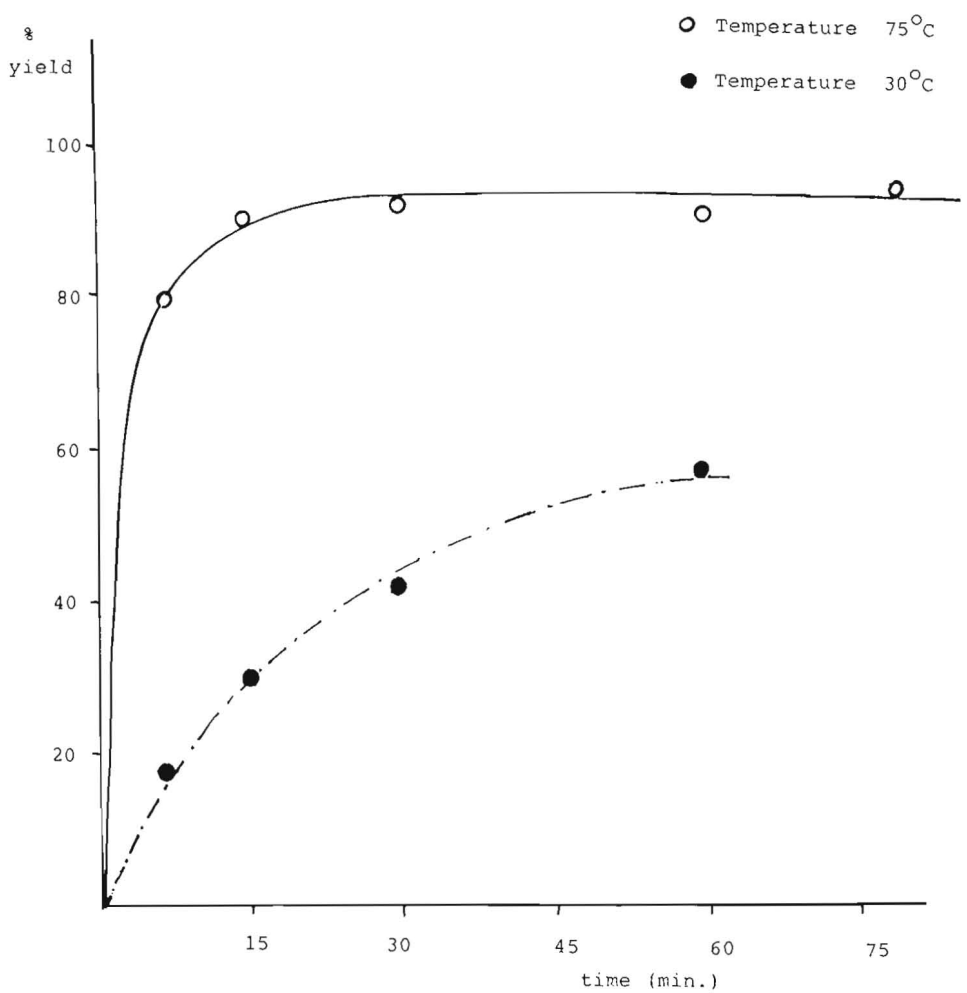

Fig. 1. Rate of exchange of R.B. in 96% Ethanol-CCl_4 at different temperatures.

of exchange within one hour reaction time. Excess of inactive iodine may reduce the yield by isotopic dilution or by change of original pure rose bengal to other species. Exchange with carrier free radioiodine gave the lowest yield; this may be due to adsorption or to reduction effects in the system.

C) *Effect of pH of the Solvent*

The lactone form of rose bengal proved to give the lowest yield of exchange while the monosodium salt gave the best results (better than the disodium salt).

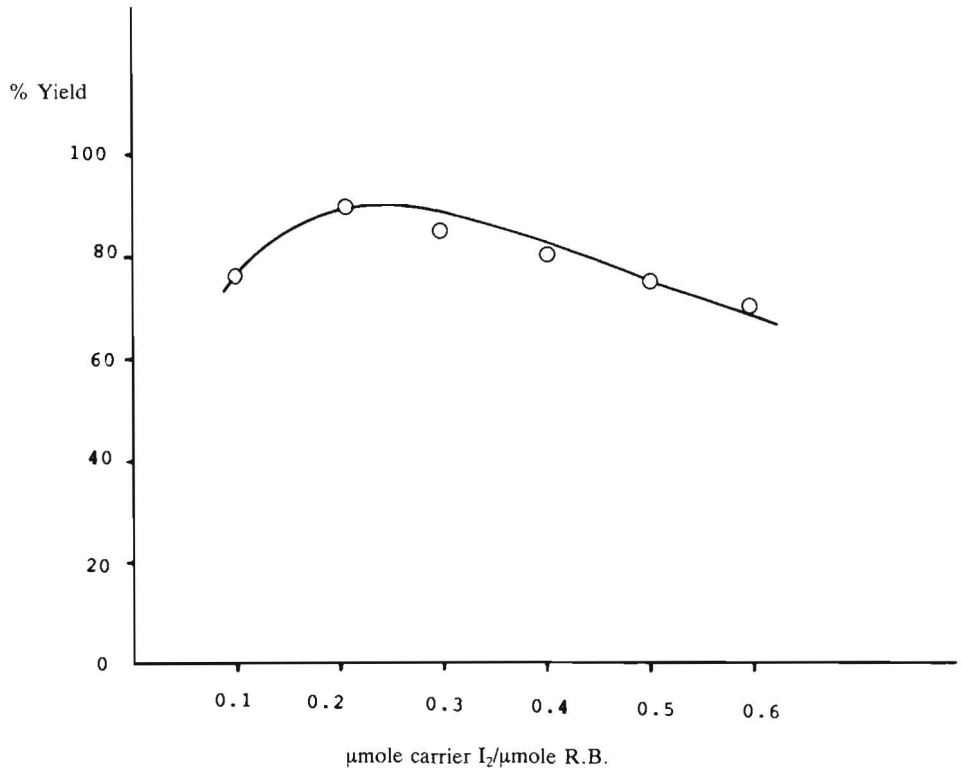

Fig. 2. Effect of carrier iodine on the exchange yield.

With respect to the reactivity of iodine atoms in R.B., there is a possibility that C–I bonds *ortho* to the phenolic group (Fig. 3) are more reactive than the two iodine atoms near the oxygen atom. If the phenolic group is slightly ionised, the inductive effect is greater which may explain why at low pHs the monosodium salt prevails and the yield of exchange is a maximum. If the pH is increased, a decrease in free iodine occurs and the exchange yield is lowered, as in the case of the disodium salt. Increase of temperature and basicity (Helmkamp *et al.* 1967) decomposes HOI*, necessary for exchange to iodide and iodate:

$$I_2 + OH^- \rightarrow HOI^* + I^-$$

$$3\ HOI^* + 3\ OH^- \rightarrow 2\ I^- + IO_3^- + 3H_2O$$

D) *Solvent Effect*

The mixed organic solvent used in this exchange reaction is 10 ml of 96% doubly distilled ethyl alcohol, in which the rose bengal is dissolved, and 3 ml of

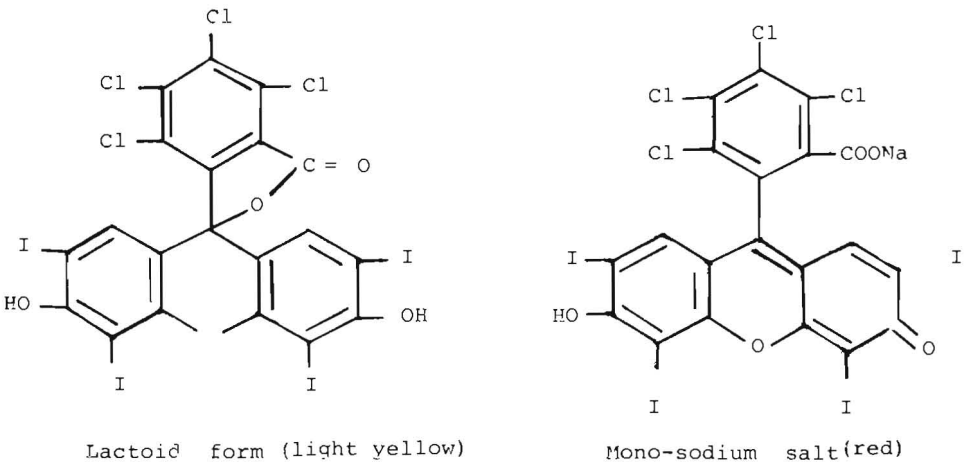

Fig. 3. Structural formulae of R.B.

inmiscible organic solvent, either ether, CCl_4 or $CHCl_3$, in which the radio and inactive iodine are extracted from aqueous solution. This immiscible organic layer was found to be the reason for the lowering of the pH of the whole solution. This lowering is more pronounced on boiling (in spite of all precautions to separate well this layer from the aqueous one). The monosodium salt of R.B. (coloured red) is stable in ether if boiled, while in CCl_4 or $CHCl_3$ on boiling it turned to the lactone colour (light yellow) and the yield of exchange dropped sharply (Fig. 4 and 5). This may be due to slight formation of H^+ in the presence of CCl_4, catalysed by I_2 on heating (Table 2). In ether, only minor reduction in pH is noticed.

To obviate this phenomenon, we added 50, 100, 250 lambdas of 0.1 N NaOH, but we found that adding 175 lambdas of 1 M acetate buffer is most appropriate and the exchange reaction goes smoother and to completion in a shorter reaction time than with ether.

Final Purification of Labelled Rose-Bengal from Free Radioiodine

The final product is obtained by slow evaporation of the solvent, dissolving the residue in 2 ml of 0.01 N NaOH, precipitating R.B. with few drops of 0.1 M HCl, centrifuging, decanting and dissolving the precipitate in 2 ml of 0.01 N NaOH. This step is repeated twice to remove any free radioiodine. The final solution is sterilized by passing it through 0.22 μm millipore filter and is ready for medical application.

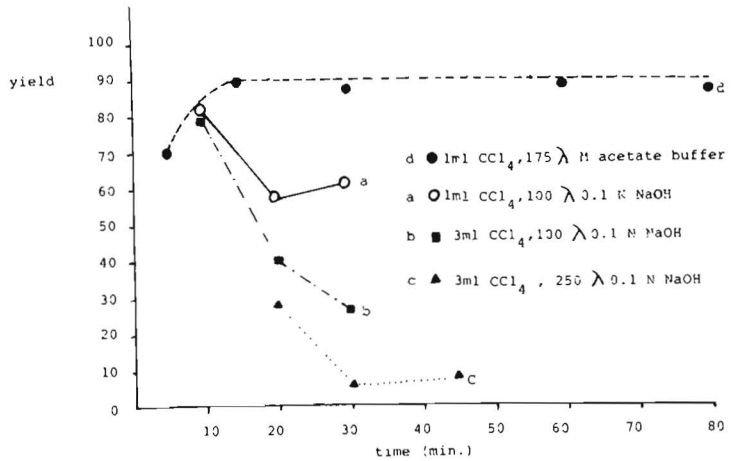

Fig. 4. Effect of pH on the yield of labelling R.B. in (Ethanol: CCl_4 at 75°).

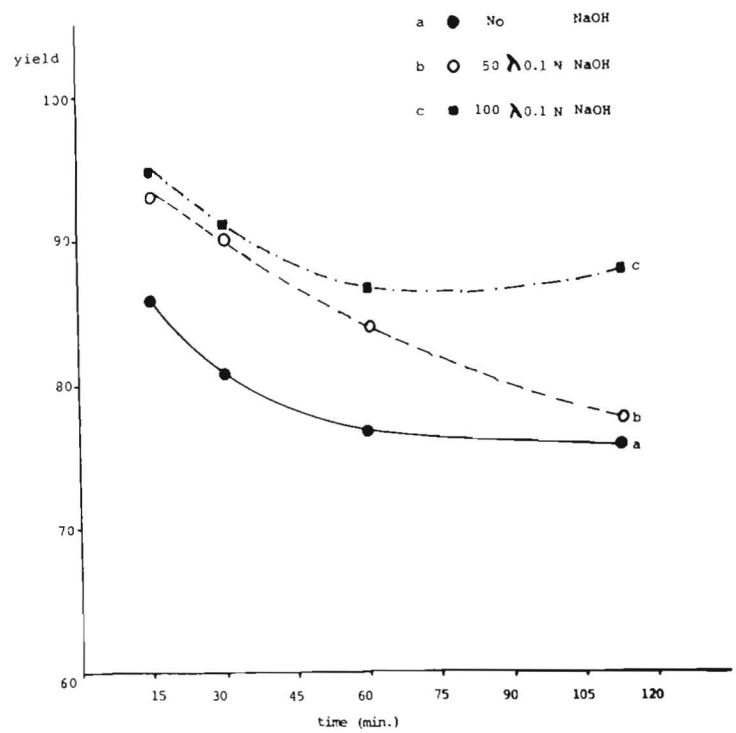

Fig. 5. Effect of pH on the yield of labelling R.B. in (Ethanol: Ether) at 75°C.

Table 2. Variation of pH with the mixture of solvents.

Solvent	0.1 N NaOH lambdas	pH value			Remarks
		initial	after 30 min	after 30 min	
1 ml CCl_4	100	5.02	1.73	–	Colour of mix. turned yellow before 30 min.
	150	5.70	2.00	–	Colour of mix. turned yellow before 30 min.
	175	6.45	3.18	1.63	Colour of mix. turned yellow before 60 min.
	200	8.90	6.00	1.80	Colour of mix. turned yellow before 60 min.
	250	11.00	–	–	Colour is kept red but low yield of exchange
3 ml CCl_4	50	5.02	1.73	–	Colour of mix. turned yellow before 30 min.
	100	5.25	1.90	–	Colour of mix. turned yellow before 30 min.
	150	5.70	2.39	–	Colour of mix. turned yellow before 30 min.
	175	6.39	1.72	–	Colour of mix. turned yellow before 30 min.
3 ml ether	25	4.40	4.05	4.20	Colour of mix. is red & no change in colour
	50	4.61	3.62	3.88	Colour of mix. is red & no change in colour
	100	4.98	3.96	4.14	Colour of mix. is red & no change in colour
	without NaOH	3.80	2.86	2.91	Change in colour after 30 min.

Conclusion

A general method for labelling Rose-Bengal in an organic medium is described. The optimum conditions for maximum yield of exchange are found to be as follows:

1) 0.2 μmole carrier inactive iodine per 1 μmole of Rose Bengal.

2) The recommended reaction mixture is 10 ml ethyl alcohol 96% as a solvent for R.B. and 3 ml of either ether or CCl_4, containing the inactive and radioiodine. In case of ether, the reaction is slow but smooth and goes to completion in two hr with a maximum exchange yield of 90% at boiling temperature. In case of CCl_4, the reaction is fast but R.B. decomposes slightly to the lactone form within 15 min with low labelling efficiency. We improved the exchange yield by adding 175 λ of 1 M acetate buffer where the labelling yield reached 90% during one hour reaction time at boiling temperature with no degradation of Rose-Bengal.

References

Desai, C.N. and **Hegesippe, M.** (1968) Preparation of Radioiodine Labelled Rose Bengal for Liver Function Studies, *Int. J. appl. Radiat. Isotopes* **19** : 153-157.

Elias, H., Arnold, C.H. and **Kloss, G.** (1973) Preparation of I^{131}-Labelled *m*-Iodohippuric Acid and Its Behaviour in Kidney Function Studies Compared to *o*-Iodohippuric Acid, *Int. J. appl. Radiat. Isotopes* **24** : 463-469.

Helmkamp, R.W., Contreras, M.A. and **Izzo, M.J.** (1967) I^{131}-Labelling of Proteins at High Activity Level with $I^{131}Cl$ Produced by Oxidation of Total Iodine in NaI^{131} Preparations, *Int. J. appl. Radiat. Isotopes* **18** : 747-754.

Hallaba, E., El-Bayoumi, S. and **El-Shaboury, G.** (1981) Preparation of High Purity Labelled Rose Bengal with Radioiodine, *J. Radioanalyt. Chem.* **65** : 171-177.

Hallaba, E. and **Raieh, M.** (1966) Production of Labelled Rose Bengal with I^{131}. *Isotopenpraxis* **4** : 194-196.

Liebster, J. and **Andrysek, O.** (1959) Labelling of Rose-Bengal with Iodine-131 by Radioactive Exchange, *Nature, London* **184** : 913.

Moore, W.J. (1965). *Physical Chemistry*, Prentice-Hall Inc., U.S.A. p. 65.

Raban, P. and **Gregora, V.** (1968) A New Method of Labelling Rose-Bengal with I^{131} or I^{125}. *Int. J. appl. Radiat. Isotopes* **19** : 361-367.

Stocklin, G. (1977) Bromine-77 and Iodine-123 Radiopharmaceuticals, *Int. J. appl. Radiat. Isotopes* **28** : 131-147.

Silver, S.M.D. (1968) *Radioactive Nuclides in Medicine and Biology*, Lea and Febiger, Philadelphia, USA, p. 353-357.

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ترقيم بعض المركبات العضوية اليودية بالتبادل الهالوجيني في وسط عضوي

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المملكة العربية السعودية

يناقش في هذا البحث الحدود المثلى لترقيم صبغة الروز بنجال ، التي تستعمل في دراسات الكبد، في مجال عضوي مؤين حيث أعطى التبادل الهالوجيني مع الروز بنجال ناتجاً مرقماً باليود المشع يزيد على ٩٠٪ في زمن لايتجاوز ساعة من التفاعل دون أي تكسير للمادة الأصلية .

ويمكن تطبيق الطريقة المستخدمة في البحث بنجاح لترقيم أية مادة عضوية غير يودية مثل الهيبوران والثير وكسين والتير وزين والأحماض الألفاتية لاستعمالها في الطب النووي .