Alkaline Incineration of Iodine-Containing Organic Material

GOSTA WIDSTROM and MONICA BJÖRKMAN

*Clinical Laboratories, Centrallasarettet,
Stocksund, Sweden*

Methods for alkaline incineration of organic material have been extensively studied as an important step in the estimation of iodine content. Alkaline incineration for analysis of protein-bound iodine, as suggested by Barker, Humphrey and Soley¹, has become generally accepted as a very useful method for estimation of circulating thyroid hormone.

In order to obtain reproducible results, all conditions in the several steps of this analysis must be kept constant within narrow limits. One reason is that it is difficult to avoid considerable losses of iodine during the ashing procedure. Difficulties are encountered both in keeping this loss constant, and in compensating for it. In experiments with radioactive iodine, van Middlesworth² found the recovery of iodine with the method of Barker *et al.*

to range from 68 to 86 %. Skanse and Hedenskog,³ using a slightly modified technique, claimed a recovery of not better than 86 %. Since the iodine recovery is evidently unsatisfactory with the incineration method in question, an attempt to reduce the losses is motivated. In the present experiments, the iodine losses were studied on dry ashing of iodine-containing substances, both protein-bound and in the iodide form, labelled with ¹³¹I.

General procedure. A working solution of Na¹³¹I, with an activity of about 0.06 μ C/ml was prepared by 500-fold dilution of a stock solution of Na¹³¹I in very dilute sulphite. Dilution was performed with 0.04 N NaOH, and NaI added as a carrier to a concentration of 20 μ g/100 ml.

Protein-bound ¹³¹I (PB¹³¹I) was obtained from patients treated for thyroid disease. The PB¹³¹I serum was diluted with normal serum to an activity convenient for measurements and treated with small charges of Dowex 2 $\times$ 8, 40 mesh, for elimination of inorganic iodine.

One ml of either of these dilute solutions and various amounts of other substances were collected in Pyrex tubes, dried overnight at 100°C, and ashed in a muffle furnace at 600°C for 3 h.

Table 1. Recovery of iodine in % after incineration at 600°C for 3 and 24 h, respectively, with various amounts of different alkalis added to the incineration mixture. Each value is the mean of 2–5 experiments.

Alkali added	Without Somogyi's reagent				With Somogyi's reagent		Precipitated with Somogyi's reagent PB ¹³¹ I 3 h
	Na ¹³¹ I		PB ¹³¹ I		Na ¹³¹ I		
	3 h	24 h	3 h	24 h	3 h	24 h	
1 ml 4 N K ₂ CO ₃	99	92	95	88	100	96	96
1 ml 4 N Na ₂ CO ₃	95	93	82	56	97	94	76
							Barker's meth.
2 ml 4 N Na ₂ CO ₃	95	94	95	78	97	95	93
1 ml 4 N NaOH + Zn	78	93	89	61	89	84	80
1 ml 4 N Na ₂ CO ₃ + S	82	66	68	39	73	19	60
1 ml 4 N NaOH + 1 ml 20 % Na ₂ S ₂ O ₅	89	85	98	70	87	91	96
1 ml 4 N NaOH + 1 ml 5 % Na ₂ S ₂ O ₅	94	95	88	63	97	89	91
1 ml 4 N NaOH + 1 ml 1 % Na ₂ S ₂ O ₅	90	72	90	49	94	69	86
2 ml 4 N CaAc ₂	13	9	18			11	2
2 ml 4 N BaAc ₂	51	9	46		60	12	40

Table 2. Recovery of iodine in % after incineration of Na^{131}I in nickel crucibles with addition of alkali acetate.

Acetate added	Metal iodide		Recovery, %			
	m.p.°C	b.p.°C	1 h	2 h	6 h	24 h
1 ml 4 N KAc	723	1 420	—	78	68	60
1 ml 4 N NaAc	651	1 300	70	66	38	20
2 ml 4 N BaAc ₂	740 d		15	8	1	0
2 ml 4 N CaAc ₂	575	718	5	2	0	0

After incineration and cooling, the ashes were dissolved in 5 ml of 1 N HCl by stirring with a glass rod, and the tubes centrifuged. Three ml of each centrifugate were taken for activity measurements, and compared with the activity of the corresponding working solution or PB^{131}I serum. An LKB scintillation counter was used for the measurements, and the activity of the samples was adjusted to give about 10 000 counts/min.

Results. Experiments in which inorganic ^{131}I and protein-bound ^{131}I , respectively, were incinerated with and without Somogyi's reagent are recorded in Table 1. When the serum protein-bound ^{131}I was incinerated together with Somogyi's reagent and 1 ml of 4 N Na_2CO_3 (method of Barker *et al.*), the mean recovery was 76 %. When K_2CO_3 was used instead of Na_2CO_3 , the recovery increased to 96 %. Substitution of K_2CO_3 for Na_2CO_3 in the incineration mixture therefore seems to be a practical means of reducing the losses of iodine during incineration*.

Table 1 shows large differences in the recovery of added iodine on incineration with various alkalis. An explanation might be provided by taking into account the melting and boiling points of the corresponding iodides. They decrease in the following order: K, Na, Ba and Ca. It is, however, difficult to predict the vapour pressure of the iodides in these somewhat complicated mixtures of salts.

* Vilkki (pers. comm. and Ref.⁴) has published a modification of Baker and Humphrey's method using KOH instead of Na_2CO_3 for the incineration, as the experience is that KOH is better than NaOH for collecting iodine in the distillation procedure. Kingsley *et al.*⁵ has substituted Na_2CO_3 for K_2CO_3 in the B. & H. incineration method because of the better purity of the potassium reagent (pers. comm.).

The following experiments were made to demonstrate the correlation between losses of iodides and their melting points on alkaline incineration. Nickel crucibles were each charged with 1 ml of Na^{131}I working solution, after which an equimolar amount of the alkali acetate of K, Na, Ca and Ba, respectively, was added. The crucibles were dried at 100°C for 24 h, and then incinerated at 600°C for 1, 2, 6 and 24 h, respectively. The ashes were brought into solution, and their activity measured as described. The results are summarized in Table 2. The correlation between the physical constants of the iodides and the losses on incineration, increasing with time, is clearly apparent.

A similar experiment, recorded in Table 3, confirms the considerable reduction in these losses when K_2CO_3 is substituted for Na_2CO_3 , as used in the alkaline incineration method of Barker *et al.* It is also fully evident that the losses were much greater in the wideopen crucibles than in the test tubes heated in an almost horizontal position.

The practical consequences of the large loss of iodide on incineration with Ca must also be taken into account. This is because the presence of Ca in normal serum might decrease the iodide recovery on incineration with Na_2CO_3 or even with K_2CO_3 . The following experiment was therefore made. One ml of Na^{131}I working solution was mixed in Pyrex tubes with 1 ml of 2 N Na_2CO_3 or K_2CO_3 , and 1 ml of CaAc_2 solution in various concentrations was added, as shown in Table 4. All the tubes were dried at 100°C for 24 h, and then incinerated at 600°C for 3 h. It can be inferred from the table that the amount of Ca present in normal serum may influence the iodide loss. The losses were considerably smaller in the K_2CO_3 than in the Na_2CO_3 series. The effect of Ca was found to be

Table 3. Recovery of iodine in % after incineration of Na¹³¹I with addition of alkali carbonate.

Carbonate added	Vessel	Iodide		1 h	Recovery, %		
		m.p.°C	b.p.°C		2 h	6 h	24 h
1 ml 4 N K ₂ CO ₃	Ni crucible	723	1 420	—	92	79	71
1 ml 4 N Na ₂ CO ₃	do.	651	1 300	81	75	70	49
1 ml 4 N K ₂ CO ₃	Pyrex tube	723	1 420	98	92	87	87
1 ml 4 N Na ₂ CO ₃	do.	651	1 300	100	92	87	64

Table 4. Effect of calcium on iodine recovery in % after alkaline incineration of Na¹³¹I.

Ca conc. mg/100 ml	K ₂ CO ₃ incineration		Na ₂ CO ₃ incineration	
	Batch 1	Batch 2	Batch 1	Batch 2
15 800	94 92	96	70 72	85
158	82 84	79	43 42	55
15.8	88 85	82	81 81	75
1.58	90 91	88	89 89	81
0	94 96	97	90 88	87

about the same in the physiologic range, but in higher concentrations the loss was much more pronounced in the Na₂CO₃ series.

It is obvious that the presence of greater concentrations of cations, the iodides of which have low meltingpoints, may result in considerable errors in the quantitative determination of iodine in organic material. The use of K₂CO₃ instead of Na₂CO₃ on incineration will result in a more complete recovery of iodine.

1. Barker, S. B., Humphrey, M. J. and Soley, M. H. *J. Clin. Invest.* **30** (1951) 55.
2. van Middlesworth *Federation Proc.* **12** (1953) 147.
3. Skanse, B. and Hedenskog, J. *Scand. J. Clin. & Lab. Invest.* **7** (1955) 291.
4. Vilkki, P. *Scand. J. Clin. & Lab. Invest.* **10** (1958) 272.
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The Oxidation of Glycosides

X. * Reduction of Some Methyl D-Oxo-glucopyranosides

OLOF THEANDER

Träkemiska Avdelningen, Svenska Träforskningsinstitutet, Stockholm, Sweden

The reduction of oxo-*cyclo*-hexane ring compounds has been widely studied and it is well known that the proportion of axial to equatorial hydroxyl groups formed is dependent upon the type of reduction, and that hindered oxo groups afford axial alcohols. The effect of hindrance is particularly strong in catalytic hydrogenation (for summary and references see Barton¹). There is usually considered to be good conformational analogy between pyrane- and *cyclo*-hexane-rings. Raymond recently² reported the results of borohydride reduction of *myo*-inosose-2 (I) and *epi*-inosose-2 (II) and compared the ratio of the inositol isomers formed with the results previously obtained by Posternak, using catalytic hydrogenation (Pt-catalyst) and reduction

* Part IX. *Acta Chem. Scand.* **12** (1958) 1887.