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Note

Optimal analysis of alginates by decarboxylation*

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Analysis of alginates for uronic acid is made particularly difficult because of their insolubility, their resistance to hydrolysis, and the lack of availability of suitable L-guluronic acid standards. We now describe further improvements in the decarboxylation technique[1] for the analysis of uronic acids in alginates.

A preliminary analysis of the purified alginate samples by optical rotation measurements and by paper chromatography and electrophoresis of the hydrolysates has been reported previously[2]. Analyses of these samples by decarboxylation with 19% hydrochloric acid, before and after purification[2], gave inconsistent results (Table I).

Partial hydrolysis of alginate samples in conc. hydrochloric acid gave results (Table II) that were misleadingly low, as revealed by subsequent analysis with 57% hydriodic acid as the decarboxylation agent.

TABLE I

DECARBOXYLATION DATA[a]

Sample	*Nature*	*Moisture (%)*	*Dry-matter basis*	
			Sodium (%)	*Uronic anhydride (%)*
Keltone	As received	12.14	11.36	85.1, 77.9, 88.3
Manucol	As received	8.73	10.56	73.2, 82.4, 83.6
Guluronic block-polymer	As received	19.50	13.66	85.0, 90.5, 85.6, 87.8, 80.9
Guluronic block-polymer	Purified[b]	4.51	11.52	81.8, 87.2
Algal	Purified[b]	7.50	11.36	80.0, 87.5
Bacterial	Purified[b]	10.95	11.47	70.1, 63.4

[a]19% Hydrochloric acid for 2 h at 145°. [b]*cf.* Ref. 2.

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TABLE II

DECARBOXYLATION[a] DATA FOLLOWING PARTIAL HYDROLYSIS OF PURIFIED SAMPLES

Sample	*Moisture (%)*	*Hydrolysis time (h)*	*Dry-matter basis*	
			Sodium (%)	*Uronic anhydride (%)*
Keltone	12.30	24	11.84	85.2
		65		84.2
		90		84.7
Algal	7.5	120	11.36	77.9, 76.1
Bacterial	10.95	120	12.30	63.5, 64.6, 63.4

[a]19% Hydrochloric acid for 2 h at 145°.

TABLE III

DECARBOXYLATION WITH 57% HYDRIODIC ACID[a]

Sample	*Nature*	*Moisture (%)*	*Dry-matter basis*	
			Sodium (%)	*Uronic anhydride (%)*
Keltone	As received	12.14	11.36	88.9, 89.3, 89.4
Keltone	Purified[b]	12.30	11.84	86.9, 84.3, 87.7
Guluronic block-polymer	Purified[b]	4.51	11.52	81.3, 81.8, 83.6, 84.7
Algal	Purified[b]	8.77	11.36	82.8, 83.2

[a]For 1 h at 145°. [b]*cf.* Ref. 2.

Prior to the decarboxylation of alginates, it was shown that decarboxylation of D-glucurono-6,3-lactone with 57% hydriodic acid was complete within 45 min. Eighteen replicate analyses using 57% hydriodic acid (decarboxylation time of 1 h) and 57 replicate analyses using 19% hydrochloric acid (decarboxylation time of 2 h) showed a percentage mean-value of 97.6 ± 2 for the former, in good agreement with 99.9 ± 1.8 for the latter determinations.

Analysis of alginate samples with 57% hydriodic acid gave the results summarized in Table III. An increase in the reaction time from 1–3 h resulted in no improvement in the accountability of uronic acids. Analysis of a formulated food-product, a batter mix containing sodium alginate, using 19% hydrochloric acid (2 h at 145°) after partial hydrolysis, and 57% hydriodic acid (1 h at 145°), gave 6.2–6.7 and 5.3–5.4%, respectively, of uronic anhydride (dry-matter basis). The differences in these results might be explained by the shorter reaction time with the hydriodic acid, which minimises the risk of errors due to the presence of an unusually high proportion of non-uronic components.

Partial hydrolysis followed by decarboxylation with 19% hydrochloric acid gave reproducible results, but the results obtained were less consistent, and the procedure more complex, than for the hydriodic acid method. Decarboxylation with 57% hydriodic acid therefore appears to be the method of choice in so far as the analysis of alginates is concerned. A publication by Kosheleva *et al.*[3], which

appeared whilst this work was in progress, also described the use of 57% hydriodic acid as the decarboxylation agent coupled with a g.l.c. procedure for the measurement of carbon dioxide. The results for alginic acid (type unspecified) showed an "anhydro" uronic acid content of 82.3% with a relative standard deviation of 2%, based on 5 estimations. However, their reaction time of 30 min was found to be insufficient for the present method. The difference may be due to the large size of sample necessary for our method.

The results in Table III reflect, to some extent, the stability of the D-mannuronic acid and L-guluronic acid constituents of the alginates. Keltone (Table III, ManA/GulA ratios of 1.64 and 1.7) showed relatively higher recoveries (~89%) of uronic acid than the algal alginate and the homopolymeric guluronic acid block-polymer (~83% each) having ManA/GulA ratios of 0.47 and 0.05, respectively[2]. The relative instability of L-guluronic acid under hydrolytic conditions has been reported[4], but the possibility of degradation losses under the decarboxylation conditions has yet to be demonstrated.

EXPERIMENTAL

Materials. — The alginate samples Keltone and Manucol and the batter mix were generously provided by Dr. John A. Ziegler (Griffith's Laboratory, Scarborough, Ontario). Samples of algal SS/DJ, the bacterial S35, and a homopolymeric block-polymer of guluronic acid (ex manugel DJ) were gifts from Dr. C. J. Lawson (Philip Lyle Memorial Laboratory, Reading, Berks, England).

Methods. — The general analytical methods dealing with hydrolysis, paper chromatography, paper electrophoresis, optical rotation, purification, and moisture and ash contents have been described previously[2].

Decarboxylation. — Alginate samples (~10 mg) were decarboxylated by using the apparatus and technique described by Castagne and Siddiqui[1]. Both 19% hydrochloric and 57% hydriodic acids were used as decarboxylation agents. The volume of acid was increased from 3 to 5 ml. For partial hydrolysis, alginate samples (~10 mg) were hydrolysed in conc. hydrochloric acid (1.54 ml) by shaking at 0° for periods ranging from 24–120 h. The hydrolysed samples were transferred, and washed, with water (1.46 ml) followed by 19% hydrochloric acid (2 ml), leading to a total volume of 5 ml of 19% acid.

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