The Removal of Interfering Ions in the Determination of Betaine in Sugar-beet Juices and Plant Material

By A. CARRUTHERS, J. F. T. OLDFIELD and H. J. TEAGUE

(*British Sugar Corporation Limited, Research Laboratories, Bramcote, Nottingham*)

Betaines are shown not to be adsorbed by an intimate mixture of the hydroxide form of a quaternary amine anion-exchange resin and the hydrogen form of a carboxylic cation-exchange resin. Choline, dimethylglycine, methylglycine, amino acids and ionic substances are quantitatively adsorbed on the resin mixture. A method is described for treating sugar-beet juices or plant extracts with the resin mixture to remove interfering ions, and betaine can be determined in the treated liquor by any quantitative technique, such as precipitation by ammonium reineckate.

In the absence of interfering ions, betaine can be determined by precipitation with potassium tri-iodide,[1,2] phosphotungstic acid[3] or ammonium reineckate. None of these precipitants is specific for betaine, and complex procedures have been described[1] for removing some of the compounds that may be co-precipitated with betaine by potassium tri-iodide.

Walker and Erlandsen[4] used ammonium reineckate at pH 1 to precipitate betaine reineckate and overcame interference caused by precipitation of choline and strong nitrogenous bases by decomposing the crude reineckate with silver nitrate and titrating the acidic betaine nitrate against sodium hydroxide to pH 6. The nitrates of the stronger bases were not sufficiently acidic to be titrated at this pH. The procedure was used without pretreatment for determining betaine in sugar-beet molasses, but it was necessary to clarify beet-diffusion juice with lime before analysis. In applying this method to purified extracts of tissues of young roots and seedlings, Cromwell and Rennie[5] found that other basic substances were co-precipitated with betaine at pH 1 and that the reineckates of some of these substances produced acidic salts when treated with silver nitrate, thus being included in the determination as betaine.

Cromwell and Rennie therefore treated the crude precipitate with silver nitrate to remove reineckate ion and then boiled the solution in the presence of silver oxide to decompose most of the interfering substances. After acidification with hydrochloric acid and separation of the precipitated silver chloride, the betaine was again precipitated by ammonium reineckate and determined by a method similar to that described by Walker and Erlandsen.

In the absence of a complete analysis of plant material, the efficiency of specific chemical treatments for the removal of interfering ions cannot readily be forecast and the procedures are necessarily tedious, so that a single method for separating betaine free from other ionic species was desirable.

Betaine is adsorbed on strongly acidic cation-exchange resins,[6] but no satisfactory procedure is available to elute betaine quantitatively without also eluting amino acids. During studies of combinations of ion-exchange resins for the de-ionisation of beet extracts and process liquors, it was observed that, although almost complete de-ionisation was obtained with mixtures of sulphonic acid cation-exchange and quaternary amine anion-exchange resins, the effluent from the column contained betaine if a weakly acidic carboxylic cation-exchange resin was substituted for the sulphonic acid resin. The latter combination was therefore investigated as a possible method for removing interfering ions before the determination of betaine.

De-ionisation is more efficient with a mixture of acidic and basic resins than with separate columns, but it was found that betaine passed quantitatively through an intimate mixture of the hydroxide form of a strongly basic anion-exchange resin and the hydrogen form of a weakly acidic cation-exchange resin, *e.g.*, De-Acidite FF(OH) *plus* Amberlite IRC-50(H). The resin treatment quantitatively removed all ionic species, other than betaine, known to be present in beet liquors. Since betaine is not adsorbed on the resins, the process is not subject to the disadvantages of other ion-exchange methods, which require recovery of material from the ion-exchange column before analysis.

After removal of interfering ions by passing the solution for analysis through the resin mixture, betaine was determined in the treated liquor by Walker and Erlandsen's[4] reineckate

titration method. For beet molasses and all clarified beet process liquors, the betaine nitrogen, as determined by the above-mentioned method, is equal to the total nitrogen present in the effluent; the betaine would consequently be determined by any quantitative precipitation technique, either colorimetrically, as betaine reineckate, or as nitrogen by the Kjeldahl method.

The effluent from beet extract or unclarified raw juice contains small amounts of nitrogen in excess of the betaine nitrogen; this is probably due to the passage of traces of protein through the resin column. The excess of nitrogen in the effluent has been shown not to interfere with the reineckate - silver nitrate titrations, which, in conjunction with ion-exchange pre-treatment, can be used for unclarified biological materials.

Method

Materials and reagents—

Ion-exchange resin mixture—Convert 500 ml of De-Acidite FF (16 to 50 mesh) to the hydroxide form and 250 ml of Amberlite IRC-50 (16 to 50 mesh) to the hydrogen form by treating them, respectively, with two bed-volumes of 2 *N* sodium hydroxide and ten bed-volumes of *N* hydrochloric acid. Wash the resins free from alkali or acid, drain, and intimately mix two volumes of De-Acidite FF with one volume of Amberlite IRC-50.

Ammonium reineckate solution—Suspend 10 g of ammonium reineckate in 200 ml of water, shake for 30 minutes, filter, and adjust to pH 1 with hydrochloric acid (3 to 4 ml).

Preparation of columns—

Mix 10 ml of the resin mixture into a slurry with just sufficient water to cover the surface of the column of resin in a chromatographic tube prepared by joining a short length of 0·6-mm external diameter glass tubing to the base of a 16-mm × 150-mm test-tube. Avoid the use of an excessive amount of water in transferring the resin mixture to the tube, otherwise partial separation of the resins will occur, as the density of the cation-exchange resin is greater than that of the anion-exchange resin.

Procedure for sugar-beet process juices—

The concentration of betaine in the test solution to be placed on the column should be between 1·5 and 7·5 mg per ml. Suitable amounts are contained in raw juice, thin juice, process syrups diluted to a sucrose content of about 15 per cent. and in 5 per cent. solutions of molasses.

Transfer 10 ml of test solution to the column, and collect the effluent at the rate of about 1 ml per minute. When the liquid level has fallen to the top of the resin, wash the column with water until a total of 50 ml of effluent and washings is obtained.

Acidify a 10-ml aliquot of the combined effluent and washings with 1·0 ml of *N* hydrochloric acid, and add 10 ml of ammonium reineckate solution. Cool to 5° C, and separate the precipitated betaine reineckate on a sintered-glass crucible (porosity No. 3). Wash the precipitate free from acid with 5-ml portions of diethyl ether. Ensure that the crucible is completely free from acid by testing with moist indicator paper, since any residual acid will be determined as betaine; approximately 30 ml of ether are required for complete washing. Dissolve the precipitate in 10 ml of a 70 per cent. aqueous solution of acetone, and finally wash the crucible with 10 ml of water.

To determine betaine in the aqueous acetone solution by Walker and Erlandsen's titration method,[4] add 10 ml of a solution 0·1 *N* in both silver nitrate and sodium nitrate, stir, and filter the mixture through a small Buchner funnel. Wash the precipitated silver reineckate with 20 ml of water, and titrate the betaine nitrate in the combined filtrate and washings with 0·01 *N* sodium hydroxide to the methyl red end-point.

Procedure for plant material—

Place 50 g of chopped root or leaf and 200 ml of water in a 500-ml beaker, weigh the beaker, and boil the contents gently for 5 minutes. Restore the evaporation loss, calculated by weighing, macerate the mixture for 10 minutes in a blender, and filter. (The boiling is merely to facilitate maceration and can be omitted for chopped sugar-beet root. If beet brei is available, a cold-water extract can be prepared with a brei to water ratio of 1 to 4 and only sufficient material to yield 50 ml of filtrate.)

Place 50 to 70 ml of the plant extract on the column, and collect a total of 100 ml of effluent and washings. Evaporate a 50-ml aliquot of the combined effluent and washings

to approximately 10 ml, acidify, and determine betaine by reineckate precipitation as described above. It is desirable to precipitate 3 to 15 mg of betaine; if necessary, the amount of extract placed on the column may be increased, provided that the absorption capacity, approximately 2 millimoles of ionic material, is not exceeded and that the column is washed with not less than 30 ml of water.

RE-USE OF RESINS—

When large numbers of determinations are involved it is possible, because of the greater density of the cation-exchange resin, to separate the two resins for regeneration by backwashing in a column not less than 2 inches in diameter. This separation is not practicable with less than 1 litre of resin, and it is probably more convenient when making infrequent determinations to discard the resin mixture after use.

Amberlite IRC-50 can be obtained in the hydrogen form, so that the acid treatment of this resin is not necessary if the resin is not re-used.

RESULTS

The betaine content of a standard solution of betaine hydrochloride was checked by titration with sodium hydroxide to pH 6. Various aliquots of the solution were diluted to 10 ml and placed on a column of the mixed ion-exchange resins. The betaine contents of the 50-ml portions of effluents were determined by reineckate - silver nitrate titration; the results were—

Betaine in test solution, mg ..	13·8	13·8	27·6	27·6
Betaine found in effluent, mg ..	14·1	13·9	27·9	28·5
Recovery, %	102·2	100·7	100·1	103·3

These recoveries are of the same order as those from standard solutions without column treatment, *i.e.*, betaine is not adsorbed on the resin mixture.

Beet second-carbonatation juice (10 ml) was placed on the column. This juice contains at least twenty common amino acids, principally glutamine, glutamic acid, aspartic acid, alanine, γ-aminobutyric acid, leucine and valine. The effluent was neutral, free from potassium and sodium and formed no colour under the conditions of Moore and Stein's ninhydrin reaction, which is sensitive to 0·5 p.p.m. of α-amino nitrogen.

Choline, methylglycine and dimethylglycine were also completely adsorbed. Trigonelline (N-methyl nicotinic acid betaine) was not adsorbed and was determined quantitatively by the reineckate procedure. It was concluded that the column treatment effectively removed all potential interfering ions except betaines. No betaines other than trimethylglycine have been reported in sugar-beet juices or extracts.

The recovery of standard betaine hydrochloride solution added to beet raw juice before ion-exchange treatment is shown in Table I.

TABLE I

RECOVERY OF ADDED BETAINE FROM BEET RAW JUICE

Sample No.	1	2
Betaine in raw juice, mg per 100 ml	143	145
Betaine added, mg per 100 ml	139	69·5
Betaine found, mg per 100 ml	285	215
Added betaine recovered, mg per 100 ml ..	142	70
Recovery, %	102	101

The betaine and total-nitrogen contents of the 50-ml effluents from a series of 10-ml samples of sugar-beet factory juices, including raw juice, second-carbonatation juice, thick juice diluted to a sucrose content of 15 per cent. and a 5 per cent. solution of molasses, are shown in Table II.

TABLE II

BETAINE AND TOTAL-NITROGEN CONTENTS OF EFFLUENTS FROM BEET PROCESS JUICES

Sample	Betaine-N found, mg	Total nitrogen found, mg

With the exceptions of those of the effluents from raw juice, the betaine and total-nitrogen contents do not differ by more than the probable error of the determinations.

The effluents from raw juice, however, contained nitrogen in excess of the betaine nitrogen. The fact that this excess of nitrogen does not interfere with the determination of betaine is shown by the results in Table III for the analysis of raw juices and the corresponding second-carbonatation juices from different factories.

TABLE III

BETAINE CONCENTRATIONS FOUND IN BEET PROCESS JUICES AND MOLASSES

	Concentration of betaine in—		
Factory	raw juice, parts per 100 parts of sucrose	second-carbonatation juice, parts per 100 parts of sucrose	molasses, parts per 100 parts of sucrose
A	1·25	1·25	4·42
B	1·20	1·22	3·93
C	1·28	1·29	4·35
D	1·18	1·21	3·98
E	1·44	1·41	4·32
F	1·28	1·30	4·00
G	1·27	1·31	3·96
H	1·30	1·27	3·39
Mean	1·28	1·28	4·04

In the production of second-carbonatation juice from raw juice, the protein and about 30 per cent. of the non-sugars are removed, but there are no significant differences in betaine contents, relative to sucrose, of raw juice and second-carbonatation juice. It is therefore unnecessary to clarify raw juice before the ion-exchange treatment.

The concentrations of betaine in samples of molasses are also shown in Table III. Since betaine is not removed in the factory processes it is possible to estimate the molasses production, relative to the sucrose intake, from the relative concentrations of betaine in raw juice and molasses.

Concentrations of betaine in molasses, as determined by Walker and Erlandsen's method[4] without treatment with the resin mixture, were from 9 to 14 per cent. higher than those found by the proposed procedure; this implies that potentially interfering ions are present in molasses.

REFERENCES

1. Blood, J. W., and Cranfield, H. T., *Analyst*, 1936, **61**, 829.
2. Reifer, I., *N.Z. J. Sci. Tech.*, 1941, **22B**, 111.
3. Davies, W. L., and Dowden, H. C., *J. Soc. Chem. Ind.*, 1936, **55**, 1755.
4. Walker, H. G., jun., and Erlandsen, R., *Anal. Chem.*, 1951, **23**, 1309.
5. Cromwell, B. T., and Rennie, S. D., *Biochem. J.*, 1953, **55**, 189.
6. Westall, R. G., *J. Sci. Food Agric.*, 1950, **1**, 191.

Received *October 14th*, 1959