Numerous methods for the quantitative analysis of sucrose have been developed. Sucrose concentration may be deter­

mined either by direct analysis of the intact sucrose or by indirect analysis. Using indirect analysis, the sucrose concentration can be quantitated by determining the concen­

tration of the hydrolysis products of sucrose -- D-glucose and/or D-fructose. In this bibliography, analysis methods using the techniques of polarimetry, isotope dilution, nuclear magnetic resonance spectroscopy, chromatography, colorimetry and spectrophotometry, enzymatic analysis, enzyme electrodes and titrimetry are summarized. It should be realized that not every method of sucrose analysis can be covered here. The coverage has been set at a level to cover methods of sucrose analysis related to sugar beet juices, in general, and to demonstrate the broad variation in the methods available for sucrose quantitative analysis and some of their problems.

Comparison of the accuracy of each individual method of sucrose analysis relative to a standard method is not straight-forward. The International Commission for Uniform Methods of Sugar Analysis (ICUMSA) has considered this question and has been unable to arrive at a con­

sistent set of conclusions. Accuracies and precisions re­

ported here are taken directly from the reference cited. No attempt is made to relate the accuracy and precision

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results for each method quantitatively to a standard method of analysis.

**POLARIMETRY (SACCHARIMETRY)**

Probably the most widely used method of sucrose analysis in the sugar industry is polarimetry (39), referred to as saccharimetry when applied to the measurement of sucrose content. This method is based on the optical activity of sucrose. Sucrose may be determined polarimetrically either with a single polarimetric measurement or with double polarimetric measurements in conjunction with sucrose inversion by acid of enzymes.

Sucrose has been determined by a single polarimetric measurement after destruction of reducing sugars (Muller Method) (38) and in the presence of invert sugar (53). Heating a sugar solution containing ethylenediamine results in the destruction of the optical activity of lactose and maltose allowing for the determination of sucrose by a single polarimetric measurement (4). Sucrose has also been determined by this method in the presence of glucose and fructose by the addition of borax (2) and boron salts (17).

The direct polarimetric measurement of a sugar solution gives the total rotation of all optically active species present, and is, consequently, a correct measure of the sucrose content only if the other substances present have no effective rotatory power. If other optically active species such as nitrogen-containing compounds (29) are present, the single polarimetric measurement must be supplemented by a second polarimetric determination. In this double polarization method, the optical activities of the impurities are kept constant while any variations in the total optical activity of the solution results from sucrose hydrolysis to invert sugar. The variation is known to be an exact function of the sucrose concentration. The hydrolysis for analytical purposes can be effected by either hydrochloric acid or the enzyme, invertase. The method of double polarimetric measurements with hydro-
chloric acid inversion is the basis of the Clerget Method (39).

Two modifications to this method pertaining solely to the hydrolysis time and temperature variations exist. These are a modification by Browne (39) recommending overnight hydrolysis at room temperature and a modification by Jackson and Gillis (23) showing inversion is complete in 8 minutes at 60°C under the conditions prescribed by Browne (39).

Nitrogen-containing compounds exhibit different rotatory power in a neutral or alkaline medium. Thus, for best results both polarizations in the double polarization methods should be performed in solutions with similar hydrogen-ion concentrations. In a promising proposal by Stanek (39), potassium citrate was added in amounts stoichiometrically equivalent to the hydrochloric acid present after acid inversion, causing the formation of potassium chloride and citric acid. To the solution with no HCl present, equivalent amounts of potassium chloride and citric acid were added, so that the two solutions were more nearly alike. Babinski and Ablamowicz (39) replaced the potassium citrate with sodium acetate. Jackson and Gillis (23) proposed two other methods (II and IV) for obtaining similar solutions. In Method II, sucrose inversion was accomplished by HCl, then neutralized with NH₄OH. To a non-inverted sucrose solution was added amounts of NH₄Cl to equal that formed in the inverted solution. In Method IV, no NH₄OH was used as in Method II and the NH₄Cl was replaced by NaCl. Method II is generally applicable. Method IV is applicable in the presence of invert sugars, but not applicable in the presence of optically active non-sugars which change rotation with acidity.

It should be realized that hydrolysis by acid requires careful temperature and time regulation. Also, hydrochloric acid is not selective, but hydrolyzes and glyco-
sidic group. Moreover, HCI influences the rotatory power of invert sugar and many other impurities occurring in natural products. For greater selectivity and no hydrolysis of impurities, the only appropriate procedure is hydrolysis by an enzyme specific for sucrose, e.g., invertase.

Sucrose determinations by double polarimetric measurements with enzymatic inversion (39) were performed by procedures similar to Browne's (39) or Jackson and Gillis' (23) modification of the Clerget Method, except invertase was used in place of hydrochloric acid for the inversion.

Specific statements about the accuracy of each of these polarimetric methods are difficult to make. In general, precision for the polarimetric analysis of pure sucrose solutions is ± 0.1% (absolute) for manual determinations and about ±0.05% (absolute) for digital determinations. Maag and Sisler (29) reported results of polarimetric analysis to be generally high by 1 to 5% (relative) compared to gas-liquid chromatography analysis (vide infra).

**ISOTOPE DILUTION**

Polarimetry is known to be unreliable in the presence of optically active non-sucrose constituents. The isotope dilution technique is not affected by interferences from other species in solution. This technique measures the yield of a non-quantitative process. A small amount of radioactive sucrose is added to a sucrose solution. After a non-quantitative purification of sucrose, the radioactivity is measured. The extent of dilution of the radio tracer indicates the amount of sucrose originally present.

Hirschmuller and coworkers (19,20,22) described the application of isotope dilution analysis to sucrose analysis in sugar beets. The method of Horning and Hirschmuller (22) required 3 to 5 days and, as such, was not useful for rapid analysis. Sibley et al. (47) improved upon the time constraints of the above method by reducing the experiment time to 24 hours by streamlining the experimental procedure. An accuracy of 0.1 to 0.2% (relative) was realized.
Mauch (32) detected a systematic error in the work of Sibley et al. (47) and corrected it by doubling the amount of water used in the digestion. Mauch found no systematic error in the work of Horning and Hirschmuller (22). Liquid scintillation counting techniques have been applied in isotope dilution studies (33) with no loss of accuracy over gas-flow proportional counters (47), but with an increase in the number of samples that can be analyzed, compared with the methods described in previous reports.

**CHROMATOGRAPHY**

Chromatographic techniques*—paper chromatography (PC), thin layer chromatography (TLC), high voltage paper electrophoresis (HVPE), ion exchange chromatography, and gas-liquid chromatography (GLC)—have not found great applicability in the quantitative analysis of sucrose solutions. The PC-Anthrone method of Sunderwirth, Olson, and Johnson (52) used descending PC with ethyl acetate-acetic acid-water (6:3:2) solvent system to easily separate 200 µg each of sucrose, glucose, and fructose. The reproducibility of the descending method was excellent using the colorimetric anthrone method for determination of the sugar. The standard deviation of the optical density for a 200 µg sucrose sample was ±1.6% (relative). This method allowed for the analysis of 20 samples in 24 hours. Trojna and Hubacek (54) separated D-glucose, D-fructose, and sucrose by PC, enzymatically inverted sucrose, then detected the invert sugars with a solution of either blue tetrazolium or blue neotetrazolium. Maximum concentrations of 42 µg D-glucose and 21 µg D-fructose per 10 milliliters of solution were determined.

Fric and Kubaniova (11) separated sucrose from glucose and fructose by PC using the solvent system butyl-alcohol acetic acid-water (4:1:5), followed by colorimetric determination of sucrose with triphenyl tetrazolium chloride. Accuracy and reproducibility of the method for two samples contain-

*Colorimetric methods used with these chromatographic techniques are discussed later.
ing 30 µg and 80 µg sucrose were 31 ±2.1 µg and 78.5 ± 3.3 µg, respectively.

Mizuna and coworkers (35) separated sugars including sucrose by PC followed by colorimetric determination using aniline hydrogen phthalate for aldoses and phoroglucinol for ketoses.

Mixtures of sucrose esters, sucrose, and raffinose have been separated by silica gel TLC on glass strips (14). Separated species were eluted from the silica gel and measured for sucrose content by the resorcinol-hydrochloric acid colorimetric method of Roe (44). Raadsveld and Klomp (43) described the determination of sucrose in the presence of glucose and lactose after separation on cellulose powder MN300 using water-ethyl acetate-pyridine (25:100:35) solvent system. The standard deviation of the sucrose analysis was ±2% (relative).

Welch and Martin (59) quantitated glucose, fructose and sucrose using TLC and densitometry. The solvent system employed was ethyl acetate-pyridine-water (8:2:1). The relative standard deviation for sucrose in the concentration range 6.00 to 14.73% was 7.2 to 15.1%.

Mabry and coworkers (30) applied HVPE to the separation of sucrose in urine samples. Quantitation was accomplished by densitometry with a standard deviation for sucrose determinations of ±9% at the 120 mg sucrose concentration level. This method allowed for the analysis of 16 to 24 samples per day.

Sinner, Simatupang and Dietrichs (50) demonstration the use of borate complex ion exchange chromatography for the separation and quantitation of sugars, including sucrose. Sugars were determined colorimetrically with 0.1% orcinol in concentrated sulfuric acid. Deviation of the individual peak areas was about ±2% (relative) for repeated injection of a sugar mixture. For quantitative measurement, 0.5 µg of
sample was used for this separation technique requiring 70 to 90 minutes per separation.

The most promising chromatographic method for sucrose analysis in sugar beet juices is the GLC method of Karr and Norman (25). Separation was accomplished on a column packed with 10% OV-17 (phenylmethyl-silicone) liquid phase on Chromosorb W, 80/100 mesh. Precision was about ±0.5% (relative) using trehalose as an internal standard. Sucrose concentrations ranged from 9.31 to 12.94 µg sucrose per 100 µg of sample with standard deviation of ±0.06 µg per 100 µg of sample.

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY
Maciel and Lowman (31) quantitated sucrose content in sugar beet juices by proton NMR. The method applied a new solvent resonance elimination technique -- Time Resolution Water Eliminated Fourier Transform (TRWEFT) NMR -- to remove the large water resonance from the proton NMR spectrum. The TRWEFT NMR technique used an added paramagnetic relaxation reagent to preferentially relax the water protons over the protons to be quantitated. The water resonance was removed from the NMR spectrum by pre-truncating the Free Induction Decay prior to calculation of the NMR power spectrum. TRWEFT NMR with an internal standard gave a linear response over the sucrose concentration range 0.0 to 0.810 M (i.e., 0.0 to 26.0 weight/weight % in water). Accuracy for sucrose analysis in sugar beet juices was 0.50% (absolute) relative to gas-liquid chromatographic analysis. Precision for one solution was better than ±0.65% (absolute) at a sucrose concentration of 15.00%.

COLORIMETRY AND SPECTROPHOTOMETRY
Sucrose in 1.0 M HCl at 80°C hydrolyzes rapidly to glucose and fructose. Fructose under the same conditions for a period of 9.25 hours produces an ultraviolet chromophore that is stable for 2 hours (12). The chromophore intensity is a direct function of the sucrose concentration and, as such, forms the basis for a simple, sensitive coloimetric
method of sucrose analysis. As little as $10^{-5}$ M sucrose can be analyzed by this method in the absence of interfering chromophores. The chromophore produced from the acid degradation of fructose was found to be hydroxymethylfurfural by thin layer chromatographic studies (12, 13). Furfurals, after formation from sugars, have also been analyzed by complex formation with azulene (46).

Analysis of small quantities of sucrose, on the order of 10-100 $\mu$g, has been performed colorimetrically by quantitation of the chromophore produced by the reaction of sucrose with anthrone (55). The chromophore formed was a complex between anthrone and the fructose moiety of sucrose. Non-reducing fructosides (e.g., raffinose, melizitose, and inulin) interfered.

The anthrone colorimetric technique has been employed in numerous other investigations involving sucrose (9, 36, 37, 40, 42, 57, 61). Accuracy to $\pm 2\%$ (relative) is obtainable for the anthrone colorimetric method for typical sugar factory samples containing about 0.2 $\mu$g of sucrose per milliliter (51).

Johnson et al. (24) combined enzymatic inversion of sucrose by invertase with the anthrone method for determination of sucrose in the presence of fructose and glucose. The reproducibility of this method for glucose and fructose was checked using 20 samples from a standard solution. The mean absorbance for 100 $\mu$g glucose was $0.2757 \pm 3.2\%$ and for 50 $\mu$g fructose was $0.2379 \pm 3.2\%$.

Fresenius and coworkers (10) immobilized enzymes for repeated in vitro analysis of sucrose. These authors immobilized the enzymes saccharase, hexokinase, phosphohexoseisomerase and glucose-6-phosphate-dehydrogenase at CNBr activated agarose. By means of this affinity absorption method, they determined the sucrose concentration of solutions in a closed system. The reduced form of nicotinamide dinucleotide phosphate measured spectrophotometrically
was regenerated by means of glutathion reductase. Standard deviation for a 7% sucrose solution was ±0.02% (absolute).

Papa and coworkers (41) quantitated sucrose concentration in the presence of glucose and fructose by examination of the differential reaction rates of sucrose reacting with ammonium molybdate. Formation of the reaction product, molybdenum blue, was followed spectrophotometrically.

Messineo and Musarra (34) described two methods for the determination of sucrose based upon chromophore formation in the reaction of the fructose moiety of sucrose with cysteine or cysteine and tryptophan. The first method is essentially a modification of Dische's cysteine reaction optimized for temperature and sulfuric acid concentration. The green chromophore formed in about 10 minutes, allowing the determination of as little as 1 µg of fructose in about 30 minutes. The second method was based upon formation of a pink chromophore by complexation of tryptophan with the fructose-cysteine hydrochloride complex formed in the first method. This second method required about 2.5 hours per sample and was twice as sensitive as the first method.

Guyot (16) applied Hessler's method of fructose analysis (18) to the analysis of sucrose solutions. Hessler's method (18) employed the colorimetric determination of the fructose complex with either p-anisidine or 3,3'-dimethoxybenzidine in 85% phosphoric acid in the presence of glucose. This analysis was good for fructose in the range 9-236 µg of fructose per gram of dry cotton boll. Guyot (16) analyzed fructose and sucrose by their reaction with p-anisidine. This reaction produced a yellow solution after 1.5 hours. The analysis scheme was good for 5-80 µg fructose or 10-160 µg sucrose. The reproducibility of the analysis was ±1-2% (relative).

Lunder (28) developed a colorimetric sucrose analysis scheme based upon reduction of cupric sulfate. Standard
solutions were prepared from Cu$_2$O and measured spectrophotometrically to obtain a standard curve over the range 100-350 mg Cu$_2$O. With this method, lactose, sucrose, maltose, and glucose were determined by spectrophotometric measurement of the Cu$_2$O precipitated after reduction of the Cu(II) salt without the need for preparing and standardizing titration solutions as Cajori (5) had to do.

In an earlier paragraph, the Kulka colorimetric method for ketopentoses and ketohexoses (27) and the orcinol-sulfuric acid method (56) for sugars were mentioned. The precision of the sucrose analysis by these methods depended upon the precision of the analysis for the monosaccharides, glucose and fructose. Typical precision for glucose analysis by the orcinol-sulfuric acid method was $\pm 1.4\%$ (relative). The Kulka method, based on the resorcinol-thiourea-HCI method of Roe (44), involved the reaction of fructose with resorcinol in HCl with a small amount of FeCl$_3$ present for color enhancement. The error for fructose analysis was $\pm 1\%$ (relative).

ENZYMATIC ANALYSIS AND ENZYME ELECTRODES
Enzymatic analysis of sucrose has been carried out directly by the action of the enzyme on sucrose or indirectly employing sucrose selective electrodes. Von Voorst (58) reported the determination of lactose, maltose, and sucrose by means of the differential action of yeast enzymes.

An O$_2$-sensing electrode in conjunction with invertase, mutarotase, and glucose oxidase was used by Satoh and coworkers (45) to analyze for sucrose in the range 0 to 10 mM. The analysis scheme, measuring O$_2$ uptake, required 3 minutes. The standard deviation for 5 mM sucrose was $\pm 7\%$ (relative).

Cordonnier and coworkers (7) developed a magnetic enzyme membrane for use in conjunction with a pO$_2$ electrode and the invertase-glucose oxidase enzyme system. The electrode response was linear over the sucrose concentration range
Cajori (5) determined sucrose after acid hydrolysis in the presence of glucose, fructose and maltose by iodometry with an accuracy within 3% (relative). Cupric hydroxide added to the sugar solution was reduced to Cu₂O by the sugar. The excess Cu(OH)₂ reacted with an excess of potassium iodide, generating I₂ which was quantitated by titration with sodium thiosulfate. Silin and Sapegina (48) determined sucrose content by the difference in the amounts of I₂ reacted before and after sucrose inversion. Raffinose caused errors in this method.

Williams et al. (60) investigated the quantitative oxidation of organic compounds including sucrose by potassium iodate in concentrated sulfuric acid. Quantitation was by sodium thiosulfate titration of the liberated I₂. The analytical accuracy was ±1-2% (relative).

Celsi and Sarrailh (6) employed a cupric-argentimetric reagent for the analysis of 5 to 10 μg sucrose by titration of the cupric ion with KSCN. A mercurimetric titration was employed by Belas and Soliman (1) for metallic mercury liberated from K₂HgI₄ after reaction with the aldehyde portion of sucrose. The titrant was sodium thiosulfate. The mean recovery of sucrose over the range of 25 to 200 mg sucrose was 96.8± 1.69%.

PHYSICAL CHEMICAL AND OTHER METHODS
Sucrose content has also been analyzed by measurement of such physical properties as solution specific gravity (3), viscosity (49), refractive index (21), and cryoscopic measurements (8). Fluorometric analysis (15) of sucrose using p-hydroxyphenyl-acetic acid as the substrate has been accomplished over the sucrose concentration range 0.3 to 3.0 μg/ml (1 to 100 μg total). The precision was about ± 1.5% (relative). The method required an initial enzymatic inversion with invertase. Katsuhiko and cowork-
ers (26) microbiologically assayed mixtures of glucose, lactose and sucrose by lactic acid bacteria.

SUMMARY REMARKS
The isotope dilution method is the ultimate method of sucrose analysis, but it is also expensive and time consuming. The GLC method of Karr and Norman is just as accurate as the isotope dilution method, easy enough to be used routinely in the laboratory, and requires about 12 minutes per sample. For routine analyses, the GLC method can replace the isotope dilution method in some cases as a means of checking other methods of sucrose analysis.

In general, sucrose analysis by polarimetry, requiring about 2 minutes per sample, gives higher percent sucrose values than analysis of the same samples by GLC. Maag and Sisler (29) showed the error in sucrose analysis from polarimetric analysis to be high by 1 to 5% (relative) compared to GLC analysis of the same samples. This is probably due to the presence of other optically active compounds in the juices. Sucrose concentration errors by TRWEFT NMR analysis are generally in the range -3.3 to 3.6% (relative) compared to GLC analysis of the same samples. Even at the present state of TRWEFT NMR methodology, analysis by the TRWEFT NMR technique is more reliable than analysis by the polarimetric method.

The invertase double polarization method may be depended upon to give reliable results. The two methods of Jackson and Gillis (II and IV) give inflated results due to the hydrolysis of the reversion products. The difference between the sucrose result by Jackson and Gillis' Method II and by the invertase method gives a relative measure of the reversion products hydrolyzed by HCl. The amino compound content can be determined by the difference in the results from Jackson and Gillis' Methods II and IV.

Accuracy and precision of the other sucrose analysis methods are generally not as good as the GLC, NMR, or isotope dilu-
tion methods. For reasons of time constraints and ease of analysis, use of the polarimetric method for routine sucrose analysis has not been replaced by the methods discussed above. In the future though, analysis of sucrose content in sugar beet juices may be performed by the GLC method or the TRWFT NMR method on a routine basis.

LITERATURE CITED


