

[CONTRIBUTION FROM THE LABORATORY OF FOODS AND SANITATION, UNIVERSITY OF WISCONSIN]

THE CUPRO-POTASSIUM CARBONATE SOLUTION IN THE DETERMINATION OF REDUCING SUGARS

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RECEIVED AUGUST 29, 1930

PUBLISHED DECEMBER 18, 1930

In view of the revival of interest¹ in the use of the so-called cupropotassium carbonate solution² in the determination of reducing sugars, there exists an evident need for more data and for a corroboration of some of those now extant. These ends have in part been approached in this Laboratory by the determination (1) of the dextrose equivalents of four modifications of this solution in which the concentration of copper sulfate is the only variant, and (2) of the levulose equivalent, as affected by change in method of reduction, of still other modifications³ containing materially less copper than any of the others and, finally, by a study of the action of dextrose upon one of the latter. The presentation of these data is the object of this communication.

Reagents

Six sugar-oxidizing solutions of the type in question were made of such concentrations of copper sulfate, potassium bicarbonate and potassium carbonate as directed by their sponsors. The observed instability^{1b,c} of four of these solutions—all statements in the literature to the contrary notwithstanding—made it necessary to prepare them in two parts each of such a concentration of copper sulfate and alkaline salts, respectively, as would produce, when used in the ratio of one volume of the first to four of the second, a reagent of the same composition as that used by its originator. Although a twenty-seven month period of observation on the stability of the fifth member³ of this group has to date brought out no visible sign of decomposition, yet because of a desire to maintain uniformity in procedure, this reagent also was made up in two parts without, however, disturbing the original proportions of its constituents. The compositions of the copper solutions which were used throughout this study are given in Table I.

In the preparation of solutions "B" of the above reagents (Ost's and Nyns' modifications) the normal carbonate was slowly added to about 700 cc. of water at, or near, its boiling point. Complete solution of this salt was then effected by the addition of the acid carbonate. The same tech-

¹ Nyns, *Sucrierie Belge*, **44**, 210 (1924); *Bull. assoc. école sup. brasserie Louvain*, **25**, 63 (1925); (a) Jackson, *J. Assocn. Official Agr. Chem.*, **9**, 178 (1926); (b) Jackson, *ibid.*, **12**, 166 (1929); (c) Schuette and Terrill, *ibid.*, **13**, 93 (1930); (d) Zerban and Sattler, *Ind. Eng. Chem., Anal. Ed.*, **2**, 307 (1930).

² For a bibliography of this solution see *J. Assocn. Official Agr. Chem.*, **13**, 97-98 (1930).

³ Beyersdorfer, *Z. Ver. deut. Zuckerind.*, **69**, 403 (1919).

TABLE I
COMPOSITION OF CUPRO-POTASSIUM CARBONATE SOLUTIONS

Sponsor	Solution A CuSO ₄ ·5H ₂ O, g. per liter	K ₂ CO ₃	Solution B KHCO ₃ g. per liter
Ost ⁴	117.5	312.5	125
Ost ⁴	87.5	312.5	125
Beyersdorfer ³	15.71	312.5	125
Beyersdorfer ³	3.14	312.5	125
Nyns ¹	75.0	312.5	125
Nyns ¹	126.5	312.5	125

nique was followed in making the Beyersdorfer modifications except that the water was brought to a temperature of only 60° before the carbonates were added in the same manner and order.

The dextrose used in this study was obtained from the Bureau of Standards (standard sample 41), and the levulose from the Eastman Kodak Company. The latter was found to have the same copper equivalent as a sample obtained from the former source.

Experimental Procedure

Exactly 20 cc. of the reducing solution, containing varying amounts of sugar, was added to 50 cc. of the freshly mixed cupro-potassium carbonate reagent combined in the proportion of 10 cc. of solution A and 40 cc. of solution B. The dextrose-copper equivalents were determined for the two solutions of Ost^{4,5} and the two of Nyns¹ by the procedure described elsewhere.^{1c,d} Reductions were carried out for two and one-half hours at a temperature of $48.9 \pm 0.1^\circ$, in stoppered Erlenmeyer flasks immersed to within 3 cm. of the top. The precipitated cuprous oxide was determined gravimetrically. Duplicate determinations run side by side checked excellently.

The levulose-copper equivalents of Beyersdorfer's stronger solution³ were determined by three methods of reduction in which time and temperature were the variants, the approximate relationship between these factors being an inverse one. The conditions were met by following the *modus operandi* of Munson and Walker,⁶ Defren⁷ and Nyns,¹ respectively, for effecting the reduction of the copper solution. The phenomenon of delayed flocculation^{1c} of part of the cuprous oxide was not detectable in this series of reductions.

Inasmuch as the graph of the observed reducing sugar-copper equivalents of the oxidizing solutions proved to be a straight line, there was derived for each a mathematical expression for the dual purpose of dis-

⁴ Ost, *Ber.*, **23**, 1035 (1890).

⁵ Ost, *Chem.-Ztg.*, **19**, 1784 (1895).

⁶ Munson and Walker, *THIS JOURNAL*, **28**, 663 (1906).

⁷ Defren, *ibid.*, **18**, 749 (1896).

tributing the experimental errors over the whole range and of calculating the sugar equivalents for any weight of copper. It is in this form that the data obtained in this investigation are recorded (Tables II and III).

TABLE II

THE DEXTROSE EQUIVALENT OF THE CUPRO-POTASSIUM CARBONATE SOLUTION

Copper content, mg. per cc.	Range of concn. of dextrose (x) in 20 cc., mg.	Equation for calculating Cu equiv.	Mean deviation $Cu_{obs.} - Cu_{calcd.}$, mg.
19.1 ¹	60-140	$0.2254x - 4.3725$	± 0.21
22.3 ⁵	60-140	$.2276x - 5.2175$	$\pm .14$
27.9 ⁴	60-140	$.2220x - 2.5700$	$\pm .47$
39.3 ¹	20-140	$.2198x - 0.6650$	$\pm .50$

x = mg. of dextrose; y = mg. of copper.

TABLE III

THE LEVULOSE EQUIVALENT OF THE CUPRO-POTASSIUM CARBONATE SOLUTION (BEYERSDORFER)

Method	Time of reduction, min.	Equation for calculating levulose equiv. (x) from Cu	Mean deviation $Cu_{obs.} - Cu_{calcd.}$, mg.
Over a free flame ⁶	6	$2.485x - 2.255$	± 0.20
In a boiling water-bath ⁷	15	$2.391x - 1.034$	$\pm .43$
In a water-bath at 48.9° ¹	120	$2.549x - 1.464$	$\pm .51$

The assertion by Nyns,¹ now known to be erroneous,^{1b,c} that the cupro-potassium carbonate solution under closely guarded conditions as to time, temperature and concentration is a selective reagent for levulose to the exclusion of dextrose, suggested the desirability of observing what action, if any, the latter has upon Beyersdorfer's alternative modification⁸ of this oxidizing agent which contains but one-fifth as much copper. To that end a series of dextrose solutions (0.3 g. in 100 cc.) of 20 cc. each was added to 50-cc. portions of the copper solution which had been mixed as above described and then brought to the reaction temperature (48.9°). The amount of copper reduced was determined at one-half hour intervals until a reaction time of two and one-half hours was reached. Data are presented in the form of a graph (Fig. 1).

Discussion

The maximum quantity of dextrose which any of the four reagents relevant to this study will oxidize is of the order of magnitude of 140 mg. On comparing the several dextrose-copper equivalents as calculated by means of the corresponding equation (Table II) it will be noted that there exists an exception to the generalization that the oxidizing power of the cupro-potassium carbonate reagent rises with an increase in the concentration of the copper. The situation which obtains is that the Ost solution⁵ in which there are 22.3 mg. of copper per cc. will oxidize less dextrose than

that one of Nyns¹ in which the concentration is less, or 19.1 mg. Inasmuch as the equilibrium conditions within this reagent are apparently not much better understood now than at the time of its introduction by Soldaini,⁸ no explanation of this phenomenon suggests itself except that it is perhaps its hydrogen-ion concentration which is the significant factor.

The application of the Ost solutions to the determination of dextrose under the unified conditions of this study is new for data obtained by their sponsor were based on a larger volume of reactants (150 cc.) when boiled for at least ten minutes. In view of the data communicated herein it appears that, as between these two reagents for the determination of dextrose under the conditions proposed by Nyns,¹ preference should be given the weaker solution⁵ because of its greater sensitiveness and the fact that the mean deviation between observed and calculated weights of reduced copper was materially less for this modification.

No claim had ever been advanced by its sponsor that the weakest copper solution³ which has been used in this study is quite indifferent in the presence of dextrose. Beyersdorfer, it is true, carried out his reductions in a short time, compensating for brevity of reaction by recourse to higher temperatures. The rather high ratio of the relative reducing effects of dextrose and levulose upon Nyns' stronger solution—10 mg. of copper was found to be equivalent to 3.5 mg. of levulose^{1c} or 48.5 mg. of dextrose⁹—raised the hope that, by applying his conditions to a reagent of still lower copper content, it might be demonstrated that it shows a selective reactivity for the latter sugar and therefore could be developed as a serviceable tool in the determination of this ketose in the presence of dextrose, a situation which obtains in the analysis of honey. This hope was not realized (Fig. 1). No reduction which is measurable by macro-chemical means had taken place at the end of the first half-hour of the reaction period, but after that it progressed arithmetically.

It is not improbable, however, that with the establishment of the above-mentioned ratio with respect to this reagent it will be possible to calculate

⁸ Soldaini, *Pubb. Instit. studi Sup. Firenzi Sez. med. chir.*, 1, 232 (1876); *Gazz. chim. ital.*, 6, 322 (1876).

⁹ This ratio is in good agreement with that reported by Jackson^{1b} who found that 13 mg. of dextrose precipitate the same weight of copper as 1 mg. of levulose.

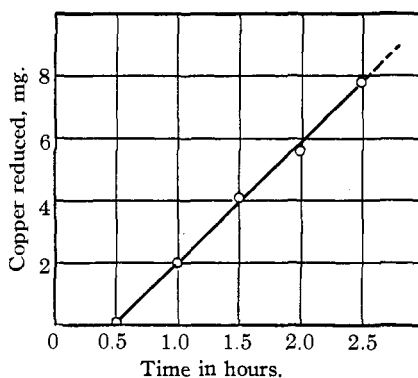


Fig. 1.—Action of dextrose upon Beyersdorfer's cupro-potassium carbonate reagent.

the percentage of these two sugars in a mixture, although such a procedure bids fair to be a laborious task, one that will involve many approximations.

Beyersdorfer's stronger reagent has not heretofore been put to a comparative test as to its levulose oxidizing powers when time and temperature of reaction are made to vary. As a result of such a study it appears (Table III) that no very material differences exist in the levulose-copper equivalents as between a reaction period of six minutes at the temperature obtainable by interposing an asbestos gauze between a beaker and a Bunsen burner,⁶ by extending that period to fifteen minutes and reducing the temperature to that of a boiling water-bath,⁷ or by allowing the reduction to proceed slowly.¹ The levulose equivalents of 10 mg. of copper are, under the above conditions and in the order given, 4.9, 4.6 and 4.5, respectively.

Choice of method of reduction will therefore be determined to some extent by (1) the smallest mean deviation between observed and calculated values, (2) convenience of operation, (3) reproducibility of results and (4) probable popularity among analysts. The mode of reduction of Munson and Walker⁶ fulfils these requirements.

Summary

In a re-investigation of the so-called cupro-potassium carbonate solution of Soldaini⁸ as modified in turn by Ost^{4,5} and by Nyns,¹ there have been obtained data on the dextrose equivalents of four forms of this reagent of a copper content lying between the limits of 19.1 and 39.3 mg. per cc. These data are presented in the form of mathematical expressions and are pertinent to a temperature of 48.9° maintained for two and one-half hours. It was also found that this sugar, under the aforesaid conditions, has a reducing action upon that modification by Beyersdorfer³ which contains but 0.8 mg. of copper per cc. This observation casts some doubt upon the acceptance of this solution as a selective reagent for levulose when both are present in the same solution.

Mathematical expressions also have been derived for calculating the levulose-copper equivalents of another reagent of this type (copper content 4.03 mg. per cc.) when either one of three modes of reduction is used, the relevant variants being time and temperature so adjusted as to bear to each other a relationship which is approximately an inverse one. It has been pointed out that there exist no very material differences in the levulose equivalents of a given weight of cuprous oxide when the latter is precipitated by either one of these procedures.

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