DETECTION AND QUANTITATIVE DETERMINATION OF SMALL AMOUNTS OF GLUCOSE IN MIXTURES CONTAINING MALTOSE

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In the analysis of hydrolytic cleavage products of glycogen or starch, such as occur in tissue extracts and in diastatic reaction products in general, we were confronted with the task of differentiating between glucose and maltose, both qualitatively and quantitatively. Our studies called for a method that permits the detection and quantitative determination of slight amounts, often but fractions of a mg., of glucose in the presence of relatively much maltose, as well as the determination of small quantities of maltose in media which contain variable quantities of glucose.

A basis for such a method was found in the great difference between the rate of fermentation of the two sugars at alkaline reaction. In a previous paper (1) we described a simple titrimetric technique which is suitable for the observation of the fermentation rate of glucose at moderately alkaline reactions. When attempting to apply the same technique to maltose, we found that, in contrast to glucose, the fermentation of this sugar is wholly suppressed when the pH of the medium is raised to from 7.5 to 8.0.

Qualitative Test for Glucose

Prepare a 20 per cent alkaline yeast suspension by rubbing up 10 gm. of commercial bakers' yeast in water and making up the volume to approximately 50 cc. Add 1 cc. of phenol red indicator (0.06 per cent aqueous solution) and 0.1 M Na₂CO₃, drop by drop with continuous stirring, until the pink color persists for about 1 minute.

Measure into a test-tube 5 cc. of the unknown (presumably mixed) sugar solution to be tested for glucose. Add 1 drop of
phenol red indicator and enough 0.01 M Na₂CO₃ to adjust the reaction to slight alkalinity (pink color, pH 7.2 to 7.4). In another test-tube of approximately the same diameter, 5 cc. of a 0.1 per cent maltose solution are made alkaline in the same manner; this serves as control.

Introduce simultaneously 5 cc. portions (use a graduated cylinder for measuring) of the slightly alkaline yeast suspension into the two test-tubes containing the unknown sugar solution and maltose, respectively. Close with rubber stoppers, mix by inversion, and allow to stand for about 5 minutes. At the end of this time run from a burette into each of the tubes 0.01 M Na₂CO₃ until the original pink color is restored. The operations in the two test-tubes should be performed as simultaneously as possible; for this reason the author prefers to use two burettes and the help of an assistant in this work. If the unknown sugar solution contains glucose, it requires in the titration distinctly more carbonate solution than the control which contains only maltose. The difference between the two titrations not only shows the presence of glucose, but, as we have shown previously (1), furnishes a fair measure of its quantity.

The presence of glucose in the solution under examination is revealed before titration, in that the pink color of the indicator fades and turns brown and yellow in it much faster than in the control tube, which contains no glucose.

The yeast suspension and carbonate solution described are suitable only when the amount of glucose in 5 cc. of solution is at least 2 mg. The smaller the quantity of glucose to be detected, the more dilute the yeast suspension and the carbonate solution must be. Thus, when the reagents are used in 10-fold dilution, i.e., a 2 per cent yeast suspension for fermentation and 0.001 M carbonate for titration, 0.5 mg. of glucose can still be detected with security, irrespective of the amount of maltose and of non-fermentable reducing matter present.

We have employed this titrimetric technique for following the rate of fermentation of various sugars at pH 7.2 to 8.0. In this procedure the control tube contains water in place of the sugar solution. As soon as the color of the indicator fades perceptibly, carbonate is added from the burette to restore the initial pink color. The control tube is treated in the same manner by an
assistant, in order to obtain correction for the self-fermentation of yeast. By reading the burettes at regular intervals, at each minute for example, figures are obtained that represent the rate of fermentation as reliably as data obtained with the aid of more elaborate apparatus. The rate of fermentation is a useful characteristic for the identification of sugars.

**Quantitative Determination**

Since pH control in the manner described while fermentation is in progress is not practicable, we examined the effect of varying Na₂CO₃, added in advance, upon the rate of fermentation of glucose and maltose. Of each of the two sugars four batches, each 200 cc., were set up with 20 gm. of washed yeast; one of each group was fermented without the addition of Na₂CO₃, the others with varying amounts of carbonate. Periodically samples were withdrawn for the determination of unfermented sugar. The results, given in Table I, show that the rate of fermentation of glucose progressively diminishes as the amount of added Na₂CO₃ is augmented. The fermentation of maltose, slow enough
in the absence of carbonate, is virtually stopped by as little as 0.03 per cent of Na₂CO₃. This, of course, is not a pattern of general validity; there are, namely, batches of yeast which have a considerable carbohydrate reserve and consequently such a vigorous self-fermentation that a small amount of carbonate is neutralized in a short time by the CO₂ derived from self-fermentation plus glucose fermentation, whereupon the fermentation of maltose begins. Fortunately, the rate of fermentation of glucose is still fast enough when Na₂CO₃ is added in an excess sufficient to safeguard the maintenance of an adequate degree of alkalinity.

**Table II**

**Selective Fermentation of Glucose in Presence of Maltose, in Solutions Containing 0.1 Gm. of Na₂CO₃ and 10 Gm. of Washed Yeast per 100 Cc., at 25°**

The sugars are given as mg. per 100 cc. of solutions; maltose is expressed as the glucose equivalent of the copper reduced by it.

<table>
<thead>
<tr>
<th>Fermentation period</th>
<th>Glucose in mixture</th>
<th>Maltose in mixture</th>
<th>Recovered as unfermented residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40.4</td>
<td>0</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>20.2</td>
<td>10.1</td>
<td>40.8</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>40.3</td>
<td>10.2</td>
</tr>
<tr>
<td>min.</td>
<td>40.2</td>
<td>20.4</td>
<td>30.6</td>
</tr>
</tbody>
</table>

In the experiment recorded in Table II we added 0.1 gm. of Na₂CO₃ to 100 cc. of sugar solution and yet, as may be seen in the first column, 40 mg. per cent of glucose were completely fermented within 30 minutes. In mixtures containing glucose and maltose in changing proportions, the glucose was completely removed and the maltose quantitatively recovered after 30 minutes of fermentation. Thus, one can allow in the selective fermentation of glucose and maltose ample safety margins both as regards the amount of added Na₂CO₃ and the time necessary for the complete fermentation of glucose.
Analytical Procedure

Into a 150 × 16 mm. Pyrex test-tube measure approximately 15 cc. of a 15 per cent suspension of washed yeast (10 gm. of yeast distributed in 100 cc. of water), centrifuge, decant, drain the supernatant water, and soak up the moisture adhering to the wall of the tube with a strip of filter paper. Introduce 15 cc. of the sugar solution, immediately followed by 1 cc. of a 1.6 per cent Na₂CO₃ solution; then stir up the yeast with a glass rod. The solution must not contain more reducing matter than corresponds, with respect to copper-reducing power, to 40 mg. per cent of glucose. Allow to ferment for 30 minutes, preventing the sedimentation of the yeast by occasional inversions of the stoppered tube. If the room temperature is below 25°, place the tube in a beaker of water with a temperature of 25–30°. Centrifuge, decant most of the clear supernatant fluid, and use 5 cc. portions for sugar determination. The difference between the reduction of the unfermented solution and the reduction after fermentation corresponds to the glucose in the mixture. The residual reduction represents maltose, provided, of course, that glucose and maltose were the sole reducing substances present. Otherwise, an additional fermentation is necessary to remove completely both the glucose and the maltose. To this end the original solution is fermented in the manner described, but without the addition of alkali, over a period of 2 to 2.5 hours. The residual reduction after this operation originates from reducing substances other than sugar, or from non-fermentable polysaccharides, or both. If A represents the total reduction of the solution, B the reduction after fermentation in alkaline medium, and C the reduction after the unmodified fermentation for 2.5 hours (non-fermentable reducing substances), then A - B = glucose and B - C = maltose.

Addition of phenol red to the sugar solution as an indicator is a useful safeguard in the process of selective fermentation. Should one encounter a batch of yeast with such an extreme degree of self-fermentation as would tend to break down within 30 minutes the safety margin of alkalinity provided in the procedure, the fading of the red color of the indicator would serve as a warning. In such instances, in order to maintain the alkalinity of the solution, Na₂CO₃ must be added, a few mg. at a time, as the need for it arises.
Copper Reagent for Determination of Slowly Oxidized Sugars

In the final step of the analysis, i.e. in the determination of the copper-reducing power of mixtures of several sugars, care must be taken that each of the sugars be as completely oxidised in the presence of the others as if it were the sole reducing substance in the solution.

It is known that the rate of oxidation of various sugars shows great differences with any given reagent, and also that increase in alkalinity accelerates the reaction (2). Thus, the oxidation of maltose is slower than that of glucose and, as will be reported later, non-fermentable reducing polysaccharides that are always present among the enzymatic cleavage products of starch and of glycogen are oxidised at even a far lower rate than maltose. In order to accomplish the complete oxidation of the slowest reacting sugar within a reasonable length of time, it was found necessary to add to the Shaffer-Somogyi series of solutions a highly alkaline copper reagent, which has the following composition:

\[
\begin{align*}
\text{Na}_2\text{CO}_3 & \quad \ldots \ldots \ldots \ldots \ldots \text{25 gm.} \\
\text{Rochelle salt} & \quad \ldots \ldots \ldots \ldots \ldots \text{25} \quad \text{“} \\
\text{NaOH, 1.0 N} & \quad \ldots \ldots \ldots \ldots \ldots \text{40 cc.} \\
\text{CuSO}_4\cdot5\text{H}_2\text{O} & \quad \ldots \ldots \ldots \ldots \ldots \text{6 gm.} \\
\text{KI} & \quad \ldots \ldots \ldots \ldots \ldots \text{5} \quad \text{“} \\
\text{Na}_2\text{SO}_4^* & \quad \ldots \ldots \ldots \ldots \ldots \text{200} \quad \text{“} \\
\text{KIO}_3, 1.0 \text{ N} & \quad \ldots \ldots \ldots \ldots \ldots \text{15 cc.}
\end{align*}
\]

* The inclusion of \(\text{Na}_2\text{SO}_4\) is of recent origin. The smallest amount of glucose that can be determined with this "high alkalinity" reagent in the absence of \(\text{Na}_2\text{SO}_4\) is 0.1 mg. By incorporation of the salt, its useful range is extended to 0.02 mg. of glucose.

The reagent is prepared as previously described (3). It is a stable solution, yet it must not be unduly exposed to air, in order to avoid the absorption of \(\text{CO}_2\) and the consequent decrease of alkalinity.

The analytical procedure to be followed with this reagent does not differ from that given for the other Shaffer-Somogyi solutions. It requires for the complete oxidation of glucose a heating period of 10 minutes, for maltose 15, for the non-fermentable polysaccharides mentioned above 20 minutes. Thus, if the last named

\[
\begin{align*}
\text{Na}_2\text{CO}_3 & \quad \ldots \ldots \ldots \ldots \ldots \text{25 gm.} \\
\text{Rochelle salt} & \quad \ldots \ldots \ldots \ldots \ldots \text{25} \quad \text{“} \\
\text{NaOH, 1.0 N} & \quad \ldots \ldots \ldots \ldots \ldots \text{40 cc.} \\
\text{CuSO}_4\cdot5\text{H}_2\text{O} & \quad \ldots \ldots \ldots \ldots \ldots \text{6 gm.} \\
\text{KI} & \quad \ldots \ldots \ldots \ldots \ldots \text{5} \quad \text{“} \\
\text{Na}_2\text{SO}_4^* & \quad \ldots \ldots \ldots \ldots \ldots \text{200} \quad \text{“} \\
\text{KIO}_3, 1.0 \text{ N} & \quad \ldots \ldots \ldots \ldots \ldots \text{15 cc.}
\end{align*}
\]
reducing substances are present in a mixture of sugars, heating for less than 20 minutes leads to erroneous results.

In Table III are given, in terms of 0.005 N thiosulfate, the reduction equivalents of known amounts of glucose after heating periods of 10 to 20 minutes; with the aid of these figures a curve can be constructed from which the glucose equivalents corresponding to any reduction (titration) value can be read off or conveniently tabulated.

**TABLE III**

*Titration Figures (Reduction Values) Corresponding to Known Amounts of Glucose Obtained with a High Alkalinity Copper Reagent*

Heating period, 20 minutes.

<table>
<thead>
<tr>
<th>Glucose in 5 cc. solution</th>
<th>Titration figures, 0.005 N thiosulfate</th>
<th>Glucose in 5 cc. solution</th>
<th>Titration figures, 0.005 N thiosulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td>cc.</td>
<td>mg.</td>
<td>cc.</td>
</tr>
<tr>
<td>0.03</td>
<td>0.15</td>
<td>0.25</td>
<td>1.52</td>
</tr>
<tr>
<td>0.05</td>
<td>0.27</td>
<td>0.30</td>
<td>1.82</td>
</tr>
<tr>
<td>0.10</td>
<td>0.58</td>
<td>0.50</td>
<td>3.12</td>
</tr>
<tr>
<td>0.15</td>
<td>0.88</td>
<td>1.00</td>
<td>6.50</td>
</tr>
<tr>
<td>0.20</td>
<td>1.15</td>
<td>2.00</td>
<td>13.17</td>
</tr>
</tbody>
</table>

**SUMMARY**

A test for the detection of small quantities of glucose in the presence of maltose and of non-fermentable reducing substances is described.

A method is given for the quantitative determination of small quantities of glucose and maltose in mixtures which contain both sugars.

A copper reagent is described which, due to its high degree of alkalinity, is suited for the analysis of sugars which have low rates of oxidation.

**BIBLIOGRAPHY**

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