Diabetes Mellitus Testing Through the use of Benedict's Quantitative Reagent

What is the optimum volume of Benedict's test that maximises reliability and validity for measuring glucose concentration?

1 Introduction

Having diabetes type I autoimmune myself, I was interested in ways that Chemistry can help with the diagnosis of diabetes. Particularly historically, as a member of my family just three generations ago died shortly after birth, suspected to be due to him having diabetes type I and being administered a sugar containing IV. With the lack of access to methods to test for diabetes in rural Brazil in the 1930s, the doctors could not have known he had diabetes.

This led me to explore chemical methods to test glucose levels for diabetes. The modern method of glucose testing involves the use of an electronic meter and test strips; where a small sample of blood reacts with the enzyme glucose oxidase, generating a small current that is read by the meter to determine glucose concentration (Nile Red, 2017). Upon further research, I found that it would be unviable for me to experiment with glucose oxidase as that is hard to obtain and use for a lab method. However, prior to the glucose oxidase test for blood, Benedict's test was used.

Benedict's test is a simple colour-based indicator of glucose concentration in urine which was used to test for diabetes in the early 20th century after being invented in 1907 (Nile Red, 2017). Not only is this accurate to the period where my family member would have lived, but also, the chemicals for creating Benedict's solution are readily available in order to carry out an experiment for this investigation.

Given the importance of medicine being easily accessible, to save/improve as many lives as possible. I decided to explore how I can optimise the cost of doing this reaction, by trying to reduce the volume of reactants needed while maintaining the reliability and validity of the glucose concentration measurement. Although this method of using Benedict's test is no longer used: as Benedict's test can only measure a limited range of glucose concentrations, is limited to measuring glucose in urine, and is impractical compared to the glucose oxidase method; I still believe that there is value in exploring such a cost-optimisation problem within medicinal chemistry.

2 Investigation

2.1 Background Information

Benedict's qualitative reagent works via a redox reaction, where reducing sugars (such as glucose) give an electron to the blue Copper(II) ions in Benedict's reagent and reduce them to red Copper(I) ions (see **Equation 1**); this causes the solution to transition from blue to red, stopping at different colours depending on the concentration as seen in **Figure 1**. Additionally, Sodium Carbonate makes the solution alkaline, which is needed for

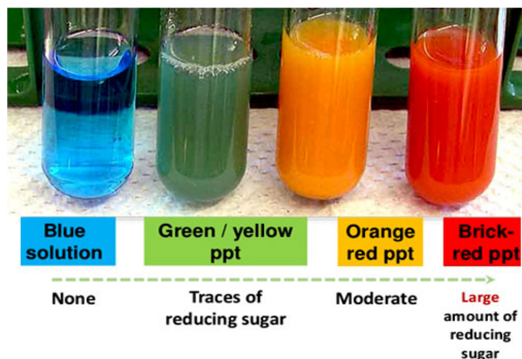


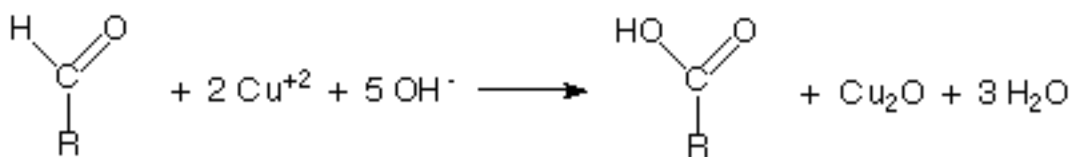
Figure 1: Colour range given by Benedict's qualitative solution (Microbiology Info, 2017).

the redox reaction. While Sodium Citrate forms a complex with Copper(II) ions to prevent their deterioration to Copper(I) in storage (Microbiology Info, 2017).

A variation, called Benedict's quantitative reagent, has a single, well-defined colour change from blue to white; which allows for a quantitative measurement of glucose concentration through a titration, over a calibrated small range of sugar concentrations. Potassium Thiocyanate is added to cause the precipitation of Cuprous Thiocyanate which is white rather than the red Copper(I) Oxide precipitate, additionally Potassium Ferricyanide is also added to prevent early oxidisation of Copper(II) ions which improves long-term storage of the solution. (HiMedia Laboratories, n.d.). This is the reaction that will be explored in this investigation as it provides quantitative results.

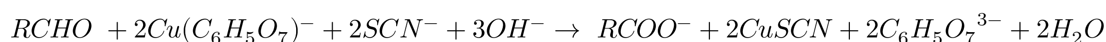
2.2 Reaction under study

Benedict's qualitative reagent follows the reaction below:



Equation 1: Redox reaction for Benedict's qualitative reagent (Microbiology Info, 2017).

However, Benedict's quantitative reagent is used in this investigation which allows for numerical data to be collected; it has additional parts cause the white precipitate to form, allowing for titration. The reaction is shown in the formula below:



$$RCHO + 2Cu(C_6H_5O_7)^- + 2SCN^- + 3OH^- \rightarrow RCOO^- + 2CuSCN + 2C_6H_5O_7{}^{3-} + 2H_2O$$

Equation 2: Balanced chemical equation for Benedict's quantitative reagent.

In this reaction, rather than forming bright red Cu_2O (Cuprous Oxide), white CuSCN (Copper Thiocyanate) is formed while removing the blue colour from the Cu ions in solution. This provides a clear, identifiable transition point for titration; the complete removal of blue colour as the solution goes white.

3 Variables

3.1 Independent Variable

Volume of Benedict's solution: The volume was chosen to be changed in order to find the optimum volume to use for reaction. This was because economically when Benedict's test was still in use, volume would have been a cost factor; which interests me to optimise. The accepted volume used is 10mL (Flinn Scientific, 2016), in this investigation smaller (and therefore economically cheaper) volumes of 1mL, 2.5mL, 5mL, 7.5mL, 10mL were explored.

3.2 Dependent Variable

Reliability of Benedict's solution: The reliability can be measured by two factors, the validity as indicated by the percentage error and the repeatability as indicated by the variance which shows how much the series of measurements varies from its mean value. Optimizing these two factors will result in the optimum volume to use.

3.3 Controlled Variables

Concentration of reactants in Benedict's solution: Because a fixed amount of sugar is required to reduce a fixed amount of Copper(II) ions (titration ratio) this must be controlled by always utilising the same mix of Benedict's solution for all tests.

Concentration of glucose test solution: In order to compare the validity and variance of the measurements made by different volumes of Benedict's solution, they must be measuring the same concentration glucose solution in order to compare measurements. The concentration used is widely accepted to calibrate Benedict's solution at 0.5% or 500mg/dl. Using real urine is unsuitable as not only is it a biohazard, but it does not have a controlled glucose concentration that would allow for comparison of the different volumes.

Titration Point: To keep consistent variance measurements and reduce the range, the endpoint of the titration should be kept the same: when the solution completely loses any blue colour and turns white.

Temperature of reaction: In order to complete and fully react, Benedict's solution needs to be heated to 100°C, which can be achieved by simply boiling the (mostly water) solution and measuring the temperature with a thermometer (Flinn Scientific, 2016).

3.3.1 Monitored Variables

Pressure of reaction: Pressure affects rate of reaction, which could lead to more glucose solution to titrate because of a delay caused by lower rate of reaction. Pressure is not measured to be controlled but assumed to be atmospheric pressure (1atm).

Surface area of reactants: It is assumed that all the reactants have fully dissolved in solution; the surface area can't be measured but it's assumed to be constant.

4 Method

The procedure consists of exploring volumes of calibrated glucose solution needed for the titration of Benedict's quantitative solution, over several volumes of Benedict's quantitative solution; looking at validity and repeatability. Two runs of data on two glucose solutions of the same concentration are performed to reduce random error.

4.1 Apparatus & Chemicals

Apparatus		Chemicals	
Name	Qty.	Name	Mass (g)
Hot Plate + Stir Bean	1	Sodium Carbonate	310
100mL Erlen-Meyer Flask	15	Sodium Citrate	100
Mass Balance (s±0.01g)	1	Copper Sulphate	9
Small Weighing Boats	6	Potassium Thiocyanate	62.5
Spoon/Spatula	6	Potassium Ferrocyanide	0.5
5mL Graduated Pipette (±0.1mL)	1	Anhydrous Glucose	2
50mL Burette (±0.05mL)	1	Distilled Water	1300
Funnel (fits Burette)	1		
500mL Bottle	1		
250mL Bottle	1		
500mL Beaker	1		
100mL Beaker	1		
50mL Beaker	11		
250mL Graduated Cylinder	1		

Additionally, a chemical disposal container is needed in order to safely dispose of the finished products, as Copper Sulphate is toxic to marine life.

Table 1: List of apparatus and chemicals needed.

4.2 Risk Assessment

Chemical	Warnings and Precautions
Sodium Carbonate Na_2CO_3	Causes skin irritation and serious eye irritation. Wash exposed skin thoroughly after handling. Wear eye protection, protective gloves. (Lab Chem, n.d.)
Copper (II) Sulphate Pentahydrate $CuSO_4$	Toxic if swallowed. Wash exposed skin thoroughly after handling. Do not eat, drink or smoke when using this product. If swallowed, rinse mouth; call poison centre. Storage: Keep away from heat, reducing agents, (strong) bases & water. (Lab Chem, n.d.)
Potassium Thiocyanate $KSCN$	Harmful if swallowed, causes skin irritation, Causes serious eye irritation. Wash exposed skin thoroughly after handling. Do no eat, drink or smoke when using this product. Wear protective gloves, eye protection. (Lab Chem, n.d.)
Potassium Ferricyanide $K_3FeC_6N_6$	Causes skin and eye irritation. Wash exposed skin thoroughly after handling. Wear eye protection, protective gloves. (Lab Chem, n.d.)
Sodium Citrate($C_6H_5Na_3O_7$), Glucose($C_6H_{12}O_6$) and Distilled Water(H_2O) pose no hazards and can therefore be omitted from warnings and precautions (Lab Chem, 2020). A chemical waste disposal container should be used to properly dispose of the products, as Copper Sulphate is toxic (especially to marine life), and Benedict's solution is basic.	

Table 2: Risk assessment with safety codes and precautions.

4.3 Set-Up

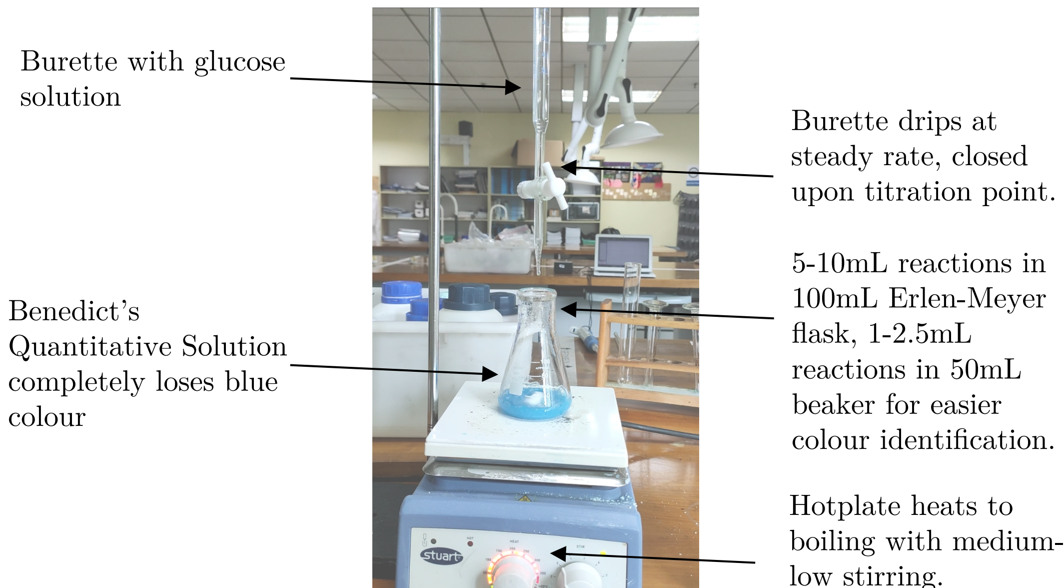


Diagram 1: Titration setup for Benedict's quantitative solution.

4.4 Preliminary Titration

One of the limitations to Benedict's test is that it is only sensitive over a small range of glucose concentrations (Flinn Scientific, 2016). In order to compensate for this a preliminary titration must be performed; if the concentration is out of range the glucose solution sample is either diluted or concentrated to ensure it is in range, this is later compensated by a dilution factor when data processing. The preliminary titration and dilution process is repeated until the sample is in the right range. This calibrated sample is then used for a whole set of 25 trials of tests over the different volumes. After collecting one run of data, a second run (another 25 trials) is collected to further reduce random error.

4.5 Experimental Procedure

1. Prepare 500mL of Benedict's quantitative solution, and store in 500mL bottle.
    a. Add 50g of Sodium Carbonate, 100g of Sodium Citrate, and 62.5g of Potassium Thiocyanate to 400mL of distilled water in a 500mL beaker. Stir & warm solution with hotplate and stir-bean until all solids dissolve. Avoid contact with chemicals, don't reuse/contaminate containers and wash hands after working.
    b. Dissolve 9g of Copper (II) Sulphate in 50mL of distilled water in a 50mL beaker, once dissolved add to the previous solution with stirring.
    c. In the same 50mL beaker, after washing, dissolve 0.5g of Potassium Ferrocyanide in 20mL of distilled water. Add this solution to the 500mL beaker and dilute the resulting solution to 500mL with distilled water.
2. Prepare 100mL of 0.5% glucose solution by dissolving 0.5g of anhydrous Glucose in 100mL of distilled water in a 100mL beaker, stir until dissolved, store in 250mL bottle.
3. Perform the **preliminary titration** to ensure the correct concentration range. (Flinn Scientific, 2016)
    a. Prepare the setup shown in **Set-Up**. Fill Burrete using the funnel with 50mL of the Glucose solution, and pipette 10mL of Benedict's solution into the 100mL Erlen-Meyer flask, adding 2g of Sodium Carbonate.
    b. Stir and heat the solution to boiling, then let the Burette drip 3mL of Glucose solution. If after a minute Benedict's solution has not completely lost its blue colour and turned white, repeat the 3mL increment until that condition is met.
    c. If colour was removed after 6mL of solution, dilute the sugar solution in half (to 200mL) and repeat **3a-3b**; If more than 12mL of solution is required to remove colour concentrate the sugar solution via evaporation to half its original volume, then repeat **3a-3b**. The final glucose solution should remove colour of 10mL of Benedict's solution with 6-12mL of glucose solution. Keep track of the dilution factor (eg. 0.5 if diluted by half, 2 if concentrated by half, 0.25 if diluted by half twice etc; will be needed later in calculations for processing)
4. Collect one trial of data (starting at a Benedict's solution volume of 10mL)
    a. Prepare the setup shown in **Set-Up**. Fill the Burrete with the funnel with 50mL of the Glucose solution and pipette the respective volume of Benedict's solution into the 100mL Erlen-Meyer flask or 50mL beaker (if volume < 5mL), adding 0.2g of Sodium Carbonate per mL of Benedict's solution. Heat solution with stirring until boiling, checking the temperature with a thermometer until it reaches 100°C. Avoid spilling the sticky boiling solution.
    b. Note the initial Burette reading (at eye-level to avoid parallax error), let the burette drip until Benedict's solution turns white, completely losing its blue colour. Top up the solution with distilled water if too much water is lost to evaporation. Note down any qualitative observations.
    c. Note the final Burette reading and write the difference between Burette readings on the respective cell in the Error! Reference source not found. table. Dispose of Benedict's solution into waste beaker as Copper Sulphate harms aquatic life.
5. Repeat step **4** four additional times to collect 5 total trials for the same volume.
6. Repeat steps **4-5** four additional times to collect a total of twenty more trials for other volumes of 7.5mL, 5mL, 2mL and 1mL respectively.
7. Repeat steps **2-6** one additional time to collect data for the second run.
8. Complete the processed data table using the calculations provided in
9. **Processed** Data on the fifty collected data points.
10. Graph; Glucose concentration vs. volume (scatter), %uncertainty vs. variance for volumes (line & bar combo) and crossing point of %uncertainty formula power approximation and variance formula linear approximation vs. volume. In order to find the optimum volume of Benedict's solution that minimises variance and %uncertainty.

5 Raw Data

Volume of Benedict's Solution (mL±0.1)	Volume of 0.5% Glucose solution to Titrate(mL±0.1)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Run 1					
1	2.6	2.1	1.6	1.6	2.6
2.5	3.9	2.8	2.8	3.5	4
5	3.2	4.6	4	4.8	5.7
7.5	5.7	6.1	6.8	6.6	7.1
10	9.8	7	6.4	8.2	8.2
Run 2					
1	1.6	1.8	1.8	1.7	1.7
2.5	3.5	4.1	4.6	3.9	3.4
5	6.3	5.4	4.4	5.3	5.8
7.5	8.6	5.8	6.9	8.4	7.2
10	9.2	7.7	8.8	8.1	9.4

Table 3: Raw data table containing 5 trials over 5 ranges, in 2 runs.

5.1 Qualitative Observations

- Benedict's solution becomes viscous if too much water evaporated.
- Some trials turned light red after white end-solution cooled down.
- Less Sodium Carbonate dissolved in smaller volumes (2.5-1mL).
- White precipitate is very fine; white when in suspension but looks more yellow when settled.

6 Processed Data

6.1 Formulas, Calculations & Error Propagation

For the *Run 1* and *Run 2* sections of the table, the values under the *Volume of 0.5% Glucose Solution to Titrate(mL)* column were calculated with the following formulas:

$$T_{Average} = \frac{T_1 + T_2 + T_3 + T_4 + T_5}{5}$$

$$T_{Range} = \frac{T_{Max} - T_{Min}}{2}$$

$$Standard\ Deviation = s = \sqrt{\frac{\sum (X - \bar{X})^2}{n-1}}$$

$$Variance = s^2$$

For the *Run 1* and *Run 2* sections of the table, the values under the *Concentration of Glucose Solution (mg/dl)* column were calculated with the following formulas:

$$Glucose\ Concentration\ (\mathrm{mg{\cdot}dl^{-1}}) = \boldsymbol{f}(V_g) = \frac{2 \cdot Volume_{Benedict's}}{Dilution\ Factor \cdot Volume_{Glucose} \div 100}$$

The columns with a blue subheading in **Table 4** indicate that the values are part of **Error Propagation** done on the concentration & volume measurements, calculated below:

$$\%_{Discrepancy} = \frac{|Glucose\ Concentration - 500|}{500}$$

$$\Delta Uncertainty_{Glucose} = \Delta Uncertainty_{Volume_{Glucose}} \cdot \left| \frac{d(Glucose\ Concentration)}{d(Volume_{Glucose})} |x = x \right|$$
$$= \Delta Uncertainty_{V_g} \cdot \left| \boldsymbol{f}'(V_g) \right|$$

$$\%Uncertainty_{Glucose} = \frac{\Delta Uncertainty_{Glucose}}{Glucose\ Concentration} \cdot 100$$

For the *Average between Runs 1 and 2* section of the table, the values are simply averaged between the values calculated for sections 1 and 2; using the formula below:

$$Value_{Average} = \frac{Value_{Run\ 1} + Value_{Run\ 2}}{2}$$

Furthermore, in order to complete the optimisation graph (**Graph 3**), a power approximation formula of the %Uncertainty $_{\text{Glucose}}$ and a linear approximation formula of the Variance $_{\text{Glucose Volume}}$ are required. These were extrapolated using a computer algebra system (Microsoft Excel) from the trendline of the respective graphs vs. Volume $_{\text{Glucose}}$, to create the formulas below:

$$\%Uncertainty_{Glucose}(V_g) = 100 \cdot 0.052 \cdot (V_g)^{-0.627}$$
$$Variance_{Glucose\ Volume}(V_g) = 0.1134 \cdot V_g$$

6.2 Sample Calculations

Sample calculations are shown for *Run 1* when *Volume of Benedict's Solution* is 1mL:

$$T_{Average} = \frac{2.6 + 2.1 + 1.6 + 1.6 + 2.6}{5}$$
$$= 2.1 \text{ mL}$$

$$T_{Range} = \frac{2.6 - 1.6}{2}$$
$$= \pm 0.5 \text{ mL}$$

Standard deviation is calculated with Microsoft Excel:

$$Standard\ Deviation = s = 0.500$$

$$Variance = s^2 = 0.500^2 = 0.250$$

$$Glucose\ Concentration\ (\text{mg}\cdot\text{dl}^{-1}) = \boldsymbol{f}(V_g) = \frac{2 \cdot 1}{0.5 \cdot 2.1 \div 100} = 190 \text{ mg}\cdot\text{dl}^{-1}$$

$$\%_{Discrepancy} = \frac{|190 - 500|}{500} = 61.90\%$$

$$\Delta Uncertainty_{Glucose} = 0.1 \cdot \left| \boldsymbol{f}' \left(\frac{2 \cdot 1}{0.5 \cdot V_g \div 100} \right) \right| = 0.1 \cdot \left| \left(\frac{-400}{2.1^2} \right) \right| = \pm 9.1 \text{ mg}\cdot\text{dl}^{-1}$$

$$\%Uncertainty_{Glucose} = \frac{9.1}{190} \cdot 100 = 4.76\%$$

And a sample of taking the average between runs, for range in this case:

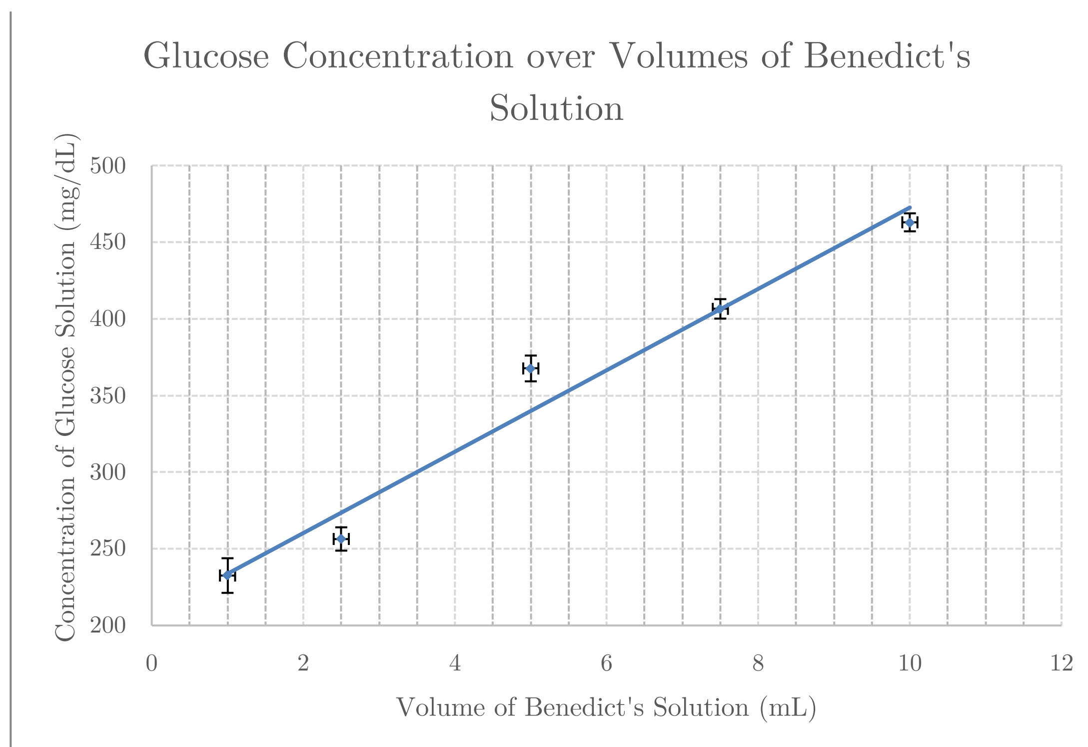
$$V_{g_{Range_{Average}}} = \frac{0.5 + 0.1}{2} = \pm 0.3 \text{ mg}\cdot\text{dl}^{-1}$$

6.3 Processed Data Table

Volume of Benedict's Solution (mL±0.1)	Volume of 0.5% Glucose Solution to Titrate(mL)				Concentration of Glucose Solution (mg/dl)			
	Avg. (mL)	Range (mL)	St. Dev.	Var.	Avg. (mg/dl)	% Dscrpy.	ΔUncer-tainty	%Uncertainty
Run 1								
1	2.1	0.5	0.500	0.250	190	61.90%	9.1	4.76%
2.5	3.4	0.6	0.579	0.335	294	41.18%	8.7	2.94%
5	4.5	1.2	0.932	0.868	448	10.31%	10.1	2.24%
7.5	6.5	0.7	0.559	0.313	464	7.12%	7.2	1.55%
10	7.9	1.7	1.308	1.712	505	1.01%	6.4	1.26%
Run 2								
1	1.7	0.1	0.084	0.007	233	53.49%	13.5	5.81%
2.5	3.9	0.6	0.485	0.235	256	48.72%	6.6	2.56%
5	5.4	1.0	0.702	0.493	368	26.47%	6.8	1.84%
7.5	7.4	1.4	1.150	1.322	407	18.70%	5.5	1.36%
10	8.6	0.9	0.723	0.523	463	7.41%	5.4	1.16%
Average between Runs 1 and 2								
1	-	0.3	0.292	0.129	212	57.70%	11.3	5.29%
2.5		0.6	0.532	0.285	275	44.95%	7.6	2.75%
5		1.1	0.817	0.681	408	18.39%	8.4	2.04%
7.5		1.1	0.855	0.818	435	12.91%	6.3	1.45%
10		1.3	1.016	1.118	484	3.20%	5.9	1.21%

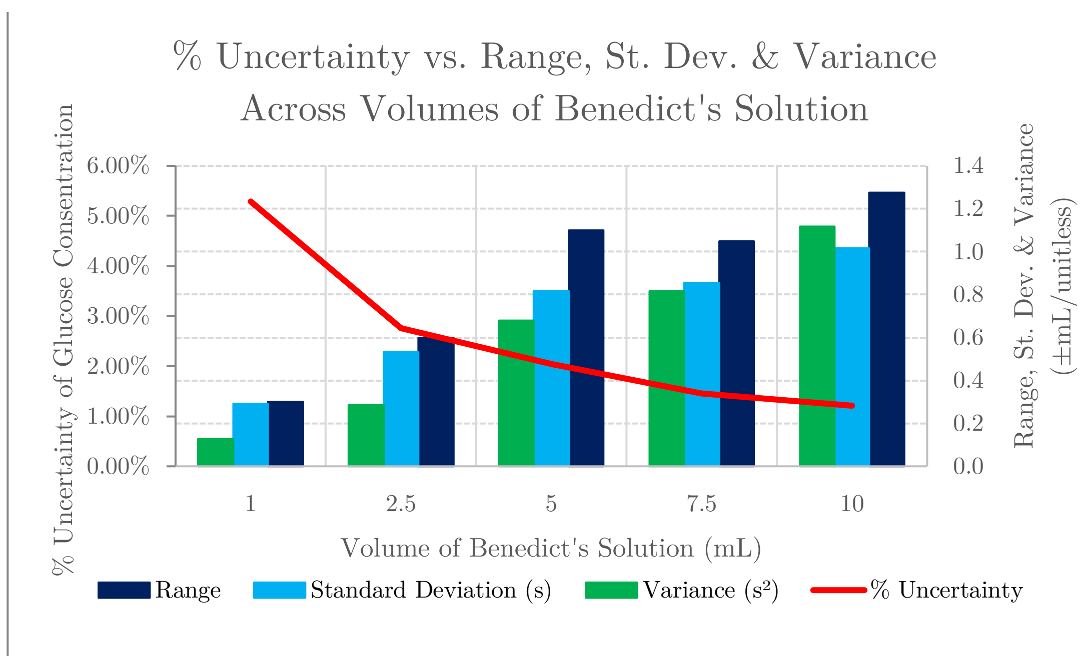
Table 4: Processed data table, analysing optimization factors for Benedict's reagent.

7 Analysis



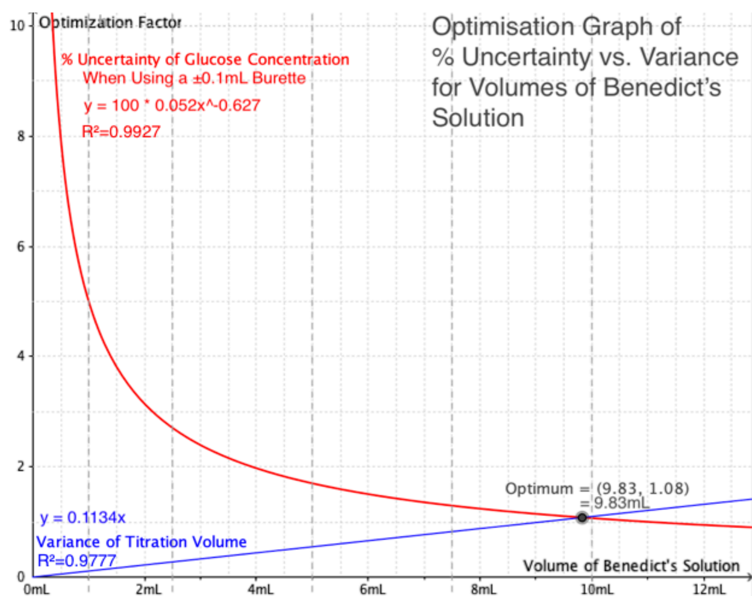
Graph 1: Average calculated glucose concentration from titration.

In Graph 1, the theoretical trend is for all trials of Benedict's test to measure the same concentration of glucose, the controlled 500mg/dl. However, the trend is not a flat line; the measured glucose concentration decreases as the volume of Benedict's solution decreases. This indicates that smaller volumes of Benedict's solution provide less valid measurements as they have a larger error from the true value. For example, for 1mL there is a percentage error of 57.6%, compared to an error of only 3.2% for a volume of 10mL. The small error bars suggest that the equipment was precise enough to collect accurate data, and that the increasing error is either systemic or simply the inherent nature of the solutions sensitivity. This increasing error is problematic as it makes lower volumes impractical due to the increasingly invalid measurements; with percentage error increasing approximately by 6% per 1mL decrease. However, there are more factors to consider.



Graph 2: Bar & Line combo graph comparing trends between % uncertainty and variance.

The red line shows us that the percentage uncertainty on the glucose measurement rises exponentially as the volume of Benedict's solution decreases, which would indicate that larger volumes of solution are preferred as they provide more certain and more valid concentration measurements. However, one must also look at the repeatability/reliability of the measurements across trials as random error randomly skews measurements. This can be measured by the range between trials for each volume, and the standard deviation and variance of each trial. The bar graph shows that there are some inconsistencies in the ranges, for example the range for 7.5mL is actually smaller than the range for 5mL which contradicts the general trend of repeatability increasing as the volume decreases. In order to mitigate this, the measure of variance can be used; variance is the standard deviation squared and it represents how much the data set varies from the mean value of that dataset. The green variance bars show a clean linear trend, with variance decreasing as volume decreases. Given that these two factors have opposite trends, this allows us to find an optimum point.



Graph 3: Optimization graph determining volume for minimum variance and % uncertainty.

Both percentage uncertainty and variance trend lines are graphed to find the optimum volume, this allows us to extrapolate values beyond our measured data. The trend for percentage uncertainty against volume of Benedict's solution is inversely exponential, as the volume decreases the percentage uncertainty increases at an increasing rate; as shown by the trend line formula of $\%_{\text{uncertainty}} = 100 \cdot 0.052 \cdot V_{\text{glucose}}{}^{-0.627}$. Additionally, this dataset has a Pearson's correlation coefficient of 0.9927; this means that there is a very strong correlation between the volume and percentage error, where within the trend line approximation, 99.27% of changes in one variable can be equated to an equivalent change in the other. On the other hand, the trend for variance over volume is linear and proportional, increasing variance at a constant rate as volume increases; with the formula $\text{Variance} = 0.1134 \cdot V_{\text{glucose}}$. Like the percentage uncertainty, the correlation is also very strong at 97.77%; which indicates that the trend line is valid and accurate at modelling the actual collected data points. Since these two trends are opposite to each other (one decreases when the other increases) there is a crossing point, which is also the optimum point; where both percentage error and variance are at their minimum. This is clearly visible on the graph as it happens when the volume of Benedict's solution is 9.83mL and the two lines cross.

8 Conclusion

The aim of this investigation was to find the smallest optimum volume of Benedict's solution that can reliably indicate glucose levels. Two runs of five trials over five different volumes (1, 2.5, 5, 7.5 and 10mL) of Benedict's solution were tested for a total of fifty tests of two 500mg/dl glucose solutions. This amount and range of data collected was sufficient to show that the optimum volume to use for Benedict's test where the percentage uncertainty and variance are minimised is 9.83mL, answering the research question. Given that the scientifically accepted volume is 10mL (Flinn Scientific, 2016), it is possible that to establish this volume as the standard, Benedict's test was already optimised for reliability and validity. This would mean that this investigation's results of 9.83mL differ from the accepted 10mL by 0.17mL or 1.7%, which is a very small error, within the precision limitations of this experiment. This brings confidence to the final result of 9.83mL presented by **Graph 3**, especially as **Graph 1** also clearly shows that higher volumes gave more valid measurements. Despite the confident result backed by various analysed data points, there is a caveat.

Because the precision of the equipment is fixed, as the volume of Benedict's test is decreased the uncertainty of measurement is inherently greater. For example, the burette has a precision of ±0.1mL, at a volume of 10mL that is only 1% uncertainty, yet at a volume of 1mL that is 10% uncertainty. This means that the percentage uncertainty trend in **Graph 3** is inherently present regardless of the nature of the reaction, it is challenging to differentiate this inherent trend from the true trend of the reaction. This may be the reason why percentage uncertainty increases exponentially rather than linearly as volume decreases; as there are two factors contributing to the increase (the fixed equipment precision and Benedict's test yielding less certain results at smaller volumes). Nonetheless, since the percentage uncertainty is not the only considered factor in finding the optimum volume; the end result remains valid. Similarly, variance can tend to be smaller with smaller volumes because the variance of a smaller volume is inherently smaller, and the equipment might not be precise enough to record it. Alas, the validity of the final 9.83mL optimum volume remains, with a small percentage error. In conclusion, although the aim of the investigation was to optimise the volume of Benedict's solution and hopefully find a smaller volume that still provides repeatable and valid results, the data shows that the optimum volume is close to 10mL at 9.83mL. Meaning that a smaller optimum volume was not found, and the aim of this investigation was unreachable, as 10mL is already the smallest optimum volume.

8.1 Evaluation

8.1.1 Strengths

One of the biggest strengths of this investigation is that fifty data points were collected, by performing ten titrations for each volume random error was greatly reduced. As shown by the small error bars on **Graph 1**, which also indicate that precise equipment was used in order to keep measurement uncertainties low. Another strength of this investigation is that preliminary titrations were performed on the glucose solution in order to ensure the glucose concentration is in a valid range for Benedict's test according to Flinn Scientific, 2016. This measure ensures that the test is able to return accurate measurements. Additionally, all the controlled variables were maintained; the same solution of Benedict's test was used for all trials within a week to ensure none of the reactants had decomposed, the glucose test solutions were kept in sealed bottles to stop evaporation that would concentrate them, and the temperature of the reaction was measured to be 100°C for every titration. This helped to reduce systemic error and keeps trials consistent. Lastly, multiple factors (volume, variance, uncertainty) were optimised in order to give the final optimum volume of 9.83mL; providing a more holistic answer to the research question.

8.1.2 Weaknesses

Despite the confident results, the investigation has many sources of error. Firstly, improper titration technique was used; five trials were simply collected without performing a rough titration which is followed by fine titrations until concurrent titres are obtained. Together with the fact that performing fifty titrations can lead to complacency and introduce human error (which caused several trials to be discarded and re-done), this greatly increased the range and random error of the trials. For example for the 1mL trial of *Run 1*, the range is particularly bad at 23.8% of the measurement (as seen in **Table 4**). This methodological issue is somewhat mitigated by averaging in data processing. Additionally, the method calls for the experimenter to prepare their own standard 0.5% glucose solution; there is no way to ensure the right concentration was prepared, this is further complicated by the concentration adjustments introduced by the preliminary titrations. This could explain systemic error

that caused all the results from Benedict's test to be below the theoretical 500mg/dl (as seen in **Graph 1**). Measuring the concentration using a modern glucometer would provide a control to truly compare the accuracy of Benedict's test practically rather than to a theoretical value. Lastly, the precision of equipment used and the quality of execution of the procedure can impact both the variance and the percentage uncertainty of the collected data, all of which would change the trends and skew the optimum point seen in **Graph 3**, and yield a different result to the investigation. Even using more precise equipment can impact the amount of variance able to be measured, particularly at small volumes. Since the experiment was only conducted once, it is challenging to place error margins on the position of that optimum point. By repeating this experiment, an average optimum point could be found that is more valid and less susceptible to random error than the one found in this investigation.

8.1.3 Limitations of scope of the investigation

A significant limitation in the scope of this investigation is that real urine samples could not be tested due to safety concerns. Urine can contain multiple kinds of reducing sugars (not just glucose) that Benedict's test would react to along with other impurities. Any monosaccharides and some disaccharides (any sugar that contains a free aldehyde) react with Benedict's test with different sensitivities, causing it to titrate at different ratios (eg. 0.0200g of glucose per 10mL of Benedict, but 0.0271g of lactose per 10mL of Benedict) (Flinn Scientific, 2016). This investigation does not consider this significant selectivity issue with Benedict's test, which is a key factor to why it was replaced and is no longer in use (Nile Red, 2017).

8.1.4 Extensions

This investigation only tried to optimise for volumes smaller than the standard 10mL used for Benedict's test in order to try to find the lowest cost Benedict's test that uses the least amount of reactants. However, to extend the investigation higher volumes of Benedict's test could be explored, to find the most accurate volume to use. Furthermore, larger volumes would fix some of the issues observed in the **Qualitative Observations**, such as: small volumes quickly evaporating and becoming viscous, and granular sodium carbonate being hard to dissolve in small volumes. Furthermore, this investigation only tested Benedict's test's properties at different volumes when measuring a single glucose concentration: 500mg/dl. It is possible that different ranges of glucose concentrations are better measured by different volumes. Creating a rough, and then fine Benedict's test procedure where a different optimum volume is chosen based on the concentration being measured. In addition, it was qualitatively observed that some trials turned red after becoming white, suggesting the composition lacked sufficient Potassium Thiocyanate which is supposed to inhibit the formation of red Copper(I) Oxide. Better compositions could be created to optimise and tweak the properties of Benedict's solution.

9 Bibliography

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