CHEMISTRY EXPERIMENTS LAB REPORT

The Technique of Volumetric Analysis

Objective

To determine the unknown concentration of NaOH using titration technique.

Introduction

A volumetric analysis's primary goal is to determine the quantity of a particular compound present in a sample, whether liquid or solid. If there are two aqueous solutions used, the concentration of one solution has to be known, and the other solution's concentration has to be quantified using titration method (Gonzalez, Jimenez and Asuero, 1990). This report involved the determination of the concentration of 20 mL NaOH via titration with 0.1M HCl.

Materials and Methods

The burette was filled up to the 0 marks with 0.1 M HCl. After that, a 20 mL volume of NaOH has dispensed onto a conical flask three drops of indicator added to the solution. The 0.1 M HCl solution was then titrated into the conical flask contents while swirling it constantly until the endpoint was reached. The volume of the endpoint volume of HCl was recorded to two decimal places. The whole procedure was repeated twice times to obtain three replicates.

Results

Standard titration of HCl and NaOH

We already know that the volume of NaOH used is a constant 20 mL. Now we need to determine the average volume of the HCl used.

Average Vol. = $\frac{20.20 + 19.20 + 18.60}{3}$ = 19.33 mL.

Replications	Initial burette reading (ml)	Final burette reading, (ml)	The volume of HCL used (Burette vol) (ml)
Replication 1	0.00	20.20	20.20
Replication 2	20.20	39.40	19.20
Replication 3	24.50	43.10	18.60

Standard deviation, σ is expressed as

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}$$

For the Replication 1, $\sigma = (20.10\text{-}19.33)^2 = 0.59$

Replication 2, $\sigma = (19.20\text{-}19.33)^2 = 0.017$

Replication 3, $\sigma = (18.60\text{-}19.33)^2 = 0.53$

Determination of the concentration of NaOH

$NaOH_{(aq)} + HCl_{(aq)} \text{ ----------> } NaCl_{(aq)} + H2O_{(l)}$

The concentration of HCl is 0.1M

No of moles of HCl = Con. X Vol in L

Convert 19.33mL to L----------> 19.33/1000 = 0.019

Moles of HCl = 0.1 x 0.019 = 0.0019 moles = No. of moles of NaOH (1:1 ratio in the reaction equation)

Therefore, the concentration of NaOH = 0.0019 moles/0.020L = 0.095 M

Discussion:

Titration can be perceived as the method used to determine either the number of moles in a given compound or the unknown concentration of a substance in a particular sample. Consequently, a chemical reaction is normally used to achieve this goal, and the reaction has to be rapid and has a quantifiable endpoint. The chemical reactions involving strong bases and acids meet these requirements, and acid-base titrations comprise the most fundamental examples of this method.

In this study, the sample is HCl as the acid and NaOH as the base where sodium hydroxide is not known. Thus, the concentration of the acid is 0.1 M, and that of the NaOH is unknown. An indicator is usually used to indicate the point which endpoint is reached. An acid-base indicator is believed to be a weak acid or base different in colour from its salt. However, at least one of them, either the indicator or it's salt needs to be strongly coloured. It is to ensure that it so that it can be seen even in strongly dilute solutions. The solution colour is, therefore, differently based on the basicity or acidity of the solution diluted in. When the acidity of acid changes significantly, a colour change is bound to take place.

In acid-base reaction equation for the given solution can be given;

$$HA + MOH \rightarrow H_2O + MA$$

Acid base water salt

The endpoint can be seen as the neutral point. The endpoint about the acid-base titration leads to salt and water production with a pH of 7. The net ionic reaction equation can be written as:

$$H^+ + OH^- \rightarrow H_2O$$

In the above experiment, the indicator that was used is phenolphthalein. If the base was to be used as the titrant, and acid as a solution in the Erlenmeyer flask, the solution would have turned to light pink after the endpoint had been reached (Janos, 2007). However, in this case, since an acid was used as the titrant, and base NaOH, was contained in the Erlenmeyer flask, the solution in the flask turns colourless after the endpoint had been reached. In this particular experiment, the chemical equation can be written as;

$$HCl + NaOH \rightarrow NaCl + H_2O$$

In this study, there are some possibilities of errors, for instance, using more than the required acid volume to titrate the NaOH solution. The volume has gone past the endpoint. Therefore the volume used would be much more than required. To address this issue, we have to do the titration slowly while firmly shaking the volumetric flask for nearly 30 seconds or until when the solutions indicate a change to colourless. The other important reasons are utilising volumetric flask that had been used with other solutions. Thus, the newest solution's concentration will most likely affect the results (Gonzalez, Jimenez and Asuero, 1990). To deal with this issue, we have to ensure that volumetric flask is spotlessly clean and properly dried. It could be the reason our result was not that precise and accurate as it should have been as can be confirmed from the standard deviation. Nevertheless, found out that the distribution of result is ranged significantly.

Conclusion:

The concentration and the number of moles of an acid or a base can be quantified using volumetric analysis, particularly the titration process. As long as one of the compounds' concentration is known, the other substance's concentration and moles can always be determined. The concentration of NaOH was found to be 0.095 M.

References

Gonzalez, G. G., Jimenez, A. M. and Asuero, A. G. (1990) ‘Titration errors in acid-base titrations’, *Microchemical Journal*, 41(1), pp. 113–120. doi: 10.1016/0026-265X(90)90103-C.

Janos, P. (2007) 'Acid-base titration curves of solid humic acids', *Reactive and Functional Polymers*, 68(1), pp. 242–247. DOI: 10.1016/ARTICLE.

Quantitative Determination of Proteins

Introduction

This experiment aimed to determine the unknown protein concentrations using the Lowry assay test (Lee et al., 2015). This entails a colourimetric analysis that quantifies the concentration based on light absorption figures detected using a spectrometer. The absorbance of a compound depends on wavelength and concentration and type of the substance. A standard curve plotted based on the absorption OD is then used to determine unknown proteins' concentration. In most cases, a series of standards stock solutions are prepared and then the graph of concentration vs OD values is plotted (Janairo et al., 2015). After determining the linear trend, an equation is generated that can be used to determine the concentration depending on the OD values.

The Lowry Protein Test largely depends on amino acids' presence, particularly the aromatic ones in the protein. A peptide bond complex is created, followed by improvement of a phosphomolybdate complex with subsequent aromatic amino acids. Therefore, this essay is often not perfectly linear and bound to be interfered by many compounds. When running the UV analysis, a wavelength of 750 nm is normally, and the sensitivity range is between 20-250 μg of assayed protein.

Materials and Methods

Reagents

Solution D

Folies Reagent

BSA stock 200 μg/mL

Unknown protein sample.

Procedure

A series of BSA dilutions from the stock solution was made, and a triplicate of the unknown protein was set. After that, 5 ml of solution D was added to each tube, vortexed and mixed well. The solution was then allowed to stand at room temperature for 10 minutes. Consequently, 0.5 ml of diluted Folin's reagent was added to all tubes and immediately vortexed. The samples were then left at room temperature for 30minutes, and the absorbance for each sample read at 750 nm. Finally, a standard curve of the standards was plotted and used to determine the unknown proteins' concentration in µg/mL.

Results

Dilution table for the preparation of standard curve for BSA using Lowry assay

NB: The final BSA concentration was determined using the formula

C1V1= C2V2

For example for 1st test tube, C1 = 200 ug/mL, V1 = 0, V2 =1 and C2=?

C2 = C1V1/V2 = 200 x 0/1= 0

BSA 200 ug/mL	Water	Final BSA conc ug/ml	Absorbance at 750 nm
0	1	0	?
0.25	0.75	50	?
0.5	0.5	100	?
0.75	0.25	150	?
1	0	200	?
Unknown A	0	?	?
Unknown B	0	?	?

The same applies to the rest of the samples/test tubes

Absorbance Values

TEST TUBE	BSA Standards Concentration	Replication1	Replication2	Replication3	Average Abs
1	0	0.013	0.015	0.016	0.015
2	50	0.255	0.248	0.261	0.255
3	100	1.600	0.412	0.423	0.812
4	150	0.527	0.545	0.549	0.540
5	200	0.697	0.670	0.706	0.691
6	Unknown A x?	0.255	0.261	0.261	0.259
7	Unknown By?	0.526	0.504	0.52	0.517

We need to calculate the average absorbance values for the three replicates for each of the samples.

The formula for calculating mean is given by;

$\mu = (\Sigma X_i) / N$

The standard deviation is given by the formula;

$\sigma = \text{sqrt} [\Sigma (X_i - \mu)^2 / N]$

So for;

Test tube 1,

Average abs = $\frac{0.013 + 0.015 + 0.016}{3}$ = 0.015

Test tube 2

Average abs = $\frac{0.255 + 0.248 + 0.261}{3}$ = 0.255

Test tube 3

Average abs = $\frac{0.600 + 0.412 + 0.423}{3}$ = 0.478

Test tube 4

Average abs = $\frac{0.527 + 0.545 + 0.549}{3}$ = 0.540

Test tube 5

Average abs = $\frac{0.697 + 0.670 + 0.706}{3}$ = 0.691

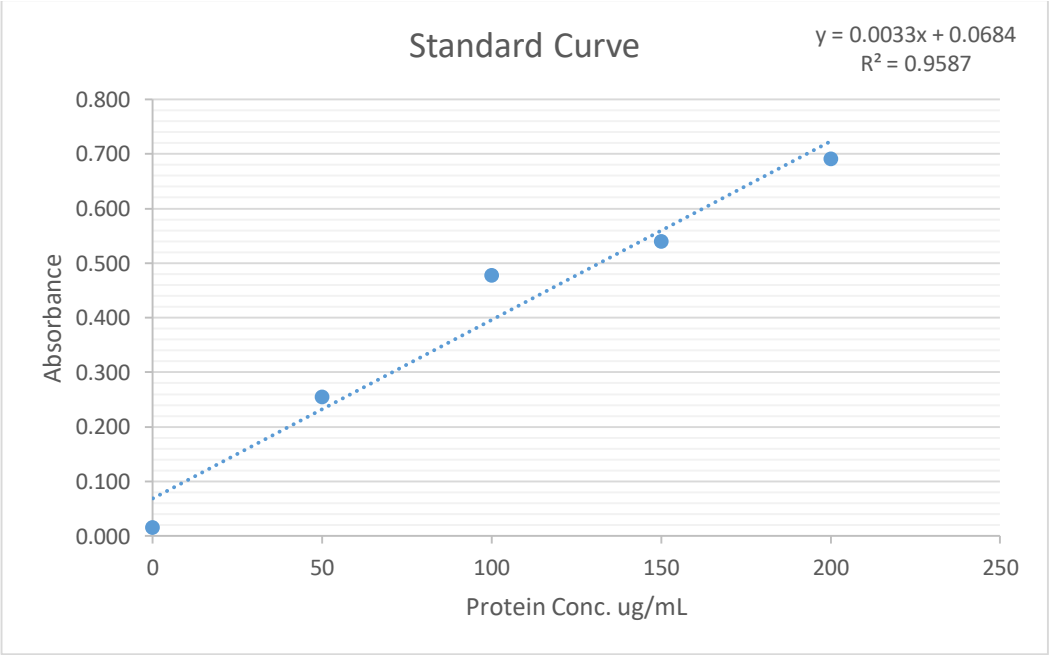
Test tube 6

Average abs = $\frac{0.255 + 0.261 + 0.261}{3}$ = 0.259

Test tube 7

Average abs = $\frac{0.526 + 0.504 + 0.520}{3}$ = 0.517

Standard Curve



The concentration of the unknown A and unknown B

From the standard curve,

Y =0.0033x + 0.0684

Where Y is the absorbance and X the concentration

Thus for unknown A;

Y=absorbance = 0.259 = 0.0033x +0.00684

0.0033x= 0.259-0.00684 =0.252

X = 0.252/0.0033 = 76.364 ~ 76 μ/mL

Therefore the concentration of the unknown A is 76 m/mL

For the unknown B;

Y=absorbance = 0.517 = 0.0033x +0.00684

0.0033x= 0.517-0.00684 =0.510

X = 0.510/0.0033 = 154.594 ~ 155 mg/mL

Therefore the concentration of the unknown B is 155 mg/mL

Discussion

The purpose of this practical was to quantify the concentration of unknown proteins in a sample by utilising the Lowry Protein Assay techniques. A linear relationship was developed by plotting the standard curve, and the equation y = 0.0033x + 0.0684 was produced. By fixing the absorbance element as y, the x, the unknown protein concentration, can be computed and quantified. The absorbance figures for the unknowns A and B were 0.259 and 0.517 respectively. The protein concentrations for the A and B were determined to be 76 and 155 μ/mL, respectively. Since the R^2 value was is 0.9587, the calculated protein concentrations may be close to the exact value (Lee et al., 2015). Nevertheless, the concentrations can still be perceived as relatively good predictions. To sum up, a different standard curve with at least a 0.99 R^2 value needs to be applied to obtain the best results. It will help in obtaining the most accurate protein concentration value.

Conclusion

This experiment aimed to assay the protein concentration of two unknown protein samples through the Lowry Protein test technique. A standard curve was plotted by using the absorbance figures of the standard protein samples. A linear relationship is was developed from the curve where the equation was found as $y = 0.033x + 0.0687$.

References

Janairo, G., Linley, M.S., Yap, L., Llanos-Lazaro, N. and Robles, J., 2015. Determination of the the sensitivity range of biuret test for undergraduate biochemistry experiments.

Lee, N., Shin, S., Chung, H.J., Kim, D.K., Lim, J.M., Park, H. and Oh, H.J., 2015. Improved quantification of protein in vaccines containing aluminium hydroxide by a simple modification of the Lowry method. *Vaccine*, *33*(39), pp.5031-5034.

Colourimetric Assay for Paracetamol

Introduction

Different organic compounds absorb different wavelengths of radiations. This kind of property characteristic of organic substances can be used to pinpoint the substance. The presence of various metal ions, or certain functional groups, for example, inorganic compounds, influences how particular substances absorb the diverse wavelengths of light, therefore causing these compounds to tend towards specific colours (Wilkinson 1976). The magnitude of absorption is also largely dependent on the concentration of the material if it is in solution form. Quantification of the amount of absorption can be utilised in determining concentrations of strongly dilute solutions (Shihana et al., 2010). Consequently, an absorption spectrometer is used to quantify how the radiation absorbed by a compound differs across the UV spectrum.
In this experiment, the popular medicinal drug paracetamol concentration was examined in a solution whereby it was reacted with nitrous acid before the solution being analysed using UV-spectrophotometer.

Materials and Methods

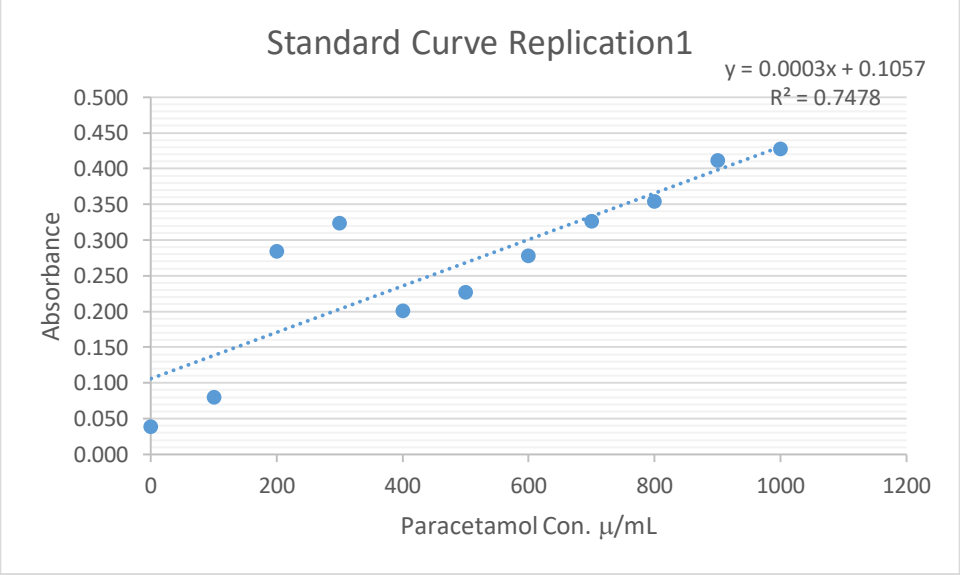
Precisely 100 mg of paracetamol was weighed and dissolved in about 10 mL of distilled water in a universal flask. The solution was then vortexed until it completely dissolved. It was transferred to a 100 mL volumetric flask and topped up with distilled water to the top and labelled as stock solution A. 1 mL aliquots of each diluted series were prepared in Eppendorf tubes from the stock solution. 100 uL of each of the standards and unknown samples were pipetted into Eppendorf tubes, and 1 mL of 3% trichloroacetic acid added the solution and vortexed. Accordingly, 300 uL of 0.07M sodium nitrite solution was added to each tube, vortexed and left to stand at room temperature for 20 minutes. %0 uLof 8M NaOH solution was

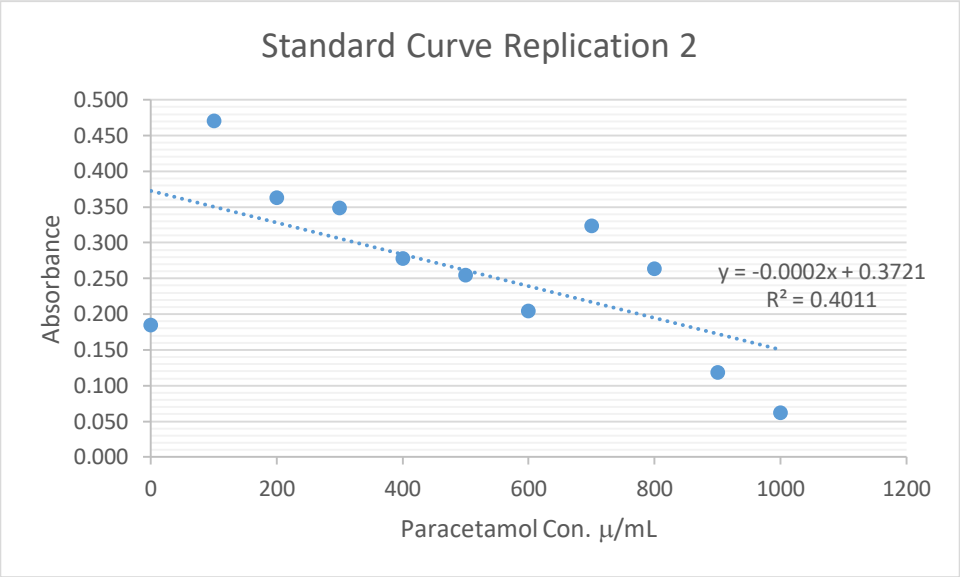
then added to each tube, capped and vortexed for 10 minutes.200 uL from each of the tubes was transferred in quadruplicates to the UV spectrophotometer, and the absorbance was read.

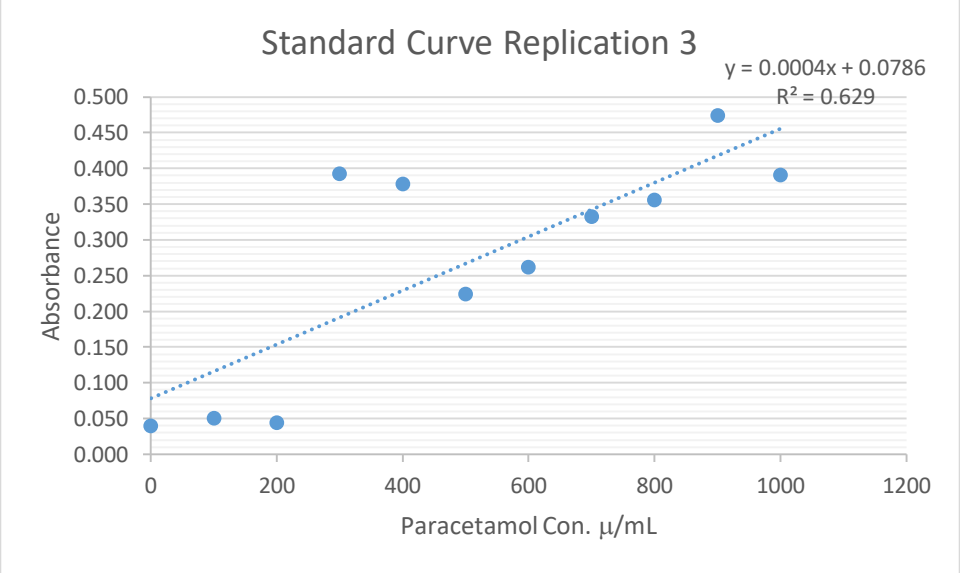
Results

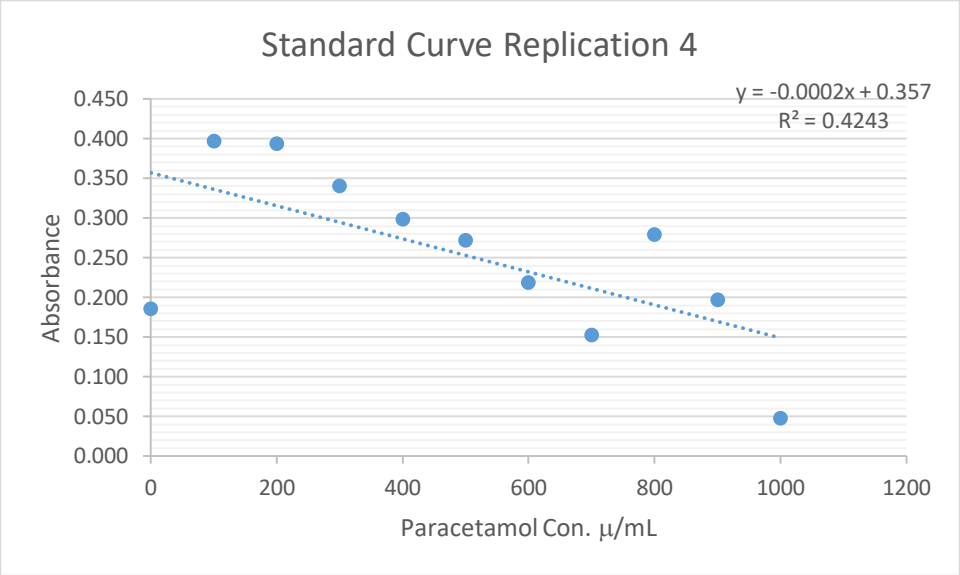
Absorbance values

	TUBE #	Paracetamol Concentration u/mL	Replication 1	Replication 2	Replication 3	Replication 4	Average Abs
	1	0	0.038	0.185	0.039	0.186	0.112
	2	100	0.080	0.471	0.050	0.397	0.249
	3	200	0.284	0.363	0.044	0.394	0.271
	4	300	0.323	0.349	0.392	0.341	0.351
	5	400	0.201	0.278	0.378	0.298	0.289
	6	500	0.226	0.255	0.224	0.272	0.244
	7	600	0.277	0.205	0.261	0.219	0.241
	8	700	0.326	0.324	0.332	0.153	0.284
	9	800	0.354	0.264	0.355	0.279	0.313
	10	900	0.411	0.119	0.474	0.197	0.300
	11	1000	0.427	0.062	0.390	0.048	0.232
	12	Unknown?	0.195	0.040	0.249	0.044	0.132




Standard Curve Replication 2
Absorbance
0.500
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0
200
400
600
800
1000
1200
Paracetamol Con. µ/mL
y = -0.0002x + 0.3721
R² = 0.4011


Standard Curve Replication 3
y = 0.0004x + 0.0786
R² = 0.629
Absorbance
0.500
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0
200
400
600
800
1000
1200
Paracetamol Con. µ/mL



Computation of the mean, the SD and % CV

The formula for determining mean is given by;

Mean = (ΣX_i) / N

The SD is given by the formula;

σ = sqrt [Σ ($X_i - \mu$)^2 / N]

While %CV is given by;

σ / μ * 100%

Mean absorbances

Flask 1

$\acute{}Mean = 0.038 \frac{+0.185 +0.039 +0.186}{4} = 0.112$

$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}}$ = Sqrt $(0.112-0.038)^2 +(0.185-0.112)^2 +(0.112-$

$0.039)^2$+ (0.1860.112)= 0.0735

%CV= σ /μ * 100% = 0.0735/0.112 * 100 = 65.23%

Flask 2

$\acute{}Mean$ = $\frac{0.080+0.471+0.050+0.397}{4}$ = 0.249

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.249-0.080)^2 + (0.471-0.249)^2 + (0.249-0.050)^2 + (0.397-0.249)^2$$

= 0.186

%CV= σ /μ * 100% = 0.186/0.249 * 100 = 74.67%

Flask 3

Mean = $\frac{0.284+0.363+0.044+0.394}{4}$ = 0.271

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.284-0.271)^2 + (0.363-0.271)^2 + (0.271-0.044)^2 + (0.394-0.271)^2$$

= 0.137

%CV= σ /μ * 100% = 0.137/0.271 * 100 = 50.55%

Flask 4

Mean = $\frac{0.323+0.349+0.392+0.341}{4}$ = 0.351

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.351-0.323)^2 + (0.351-0.351)^2 + (0.392-0.351)^2 + (0.351-0.341)^2$$

$$= 0.0253$$

$$\%CV = \sigma / \mu * 100\% = 0.0253/0.351 * 100 = 7.21\%$$

Flask 5

$$Mean = \frac{0.201+0.278+0.378+0.298}{4} = 0.289$$

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.289-0.201)^2 + (0.289-0.278)^2 + (0.378-0.289)^2 + (0.298-0.289)^2$$

$$= 0.0630$$

$$\%CV = \sigma / \mu * 100\% = 0.0630/0.289 * 100 = 21.80\%$$

Flask 6

$$Mean = \frac{0.226+0.255+0.224+0.272}{4} = 0.244$$

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.244-0.226)^2 + (0.255-0.244)^2 + (0.244-0.224)^2 + (0.272-0.244)^2$$

$$= 0.0201$$

$$\%CV = \sigma / \mu * 100\% = 0.0201/0.244 * 100 = 8.24\ \%$$

Flask 7

Mean = $0.277\underline{+0.205+0.261+0.219}{4} = 0.241$

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.277-0.241)^2 + (0.241-0.205)^2 + (0.261-0.241)^2 + (0.241-0.219)^2$$

$$= 0.0295$$

%CV= $\sigma / \mu * 100\% = 0.0295/0.241 * 100 = 12.24\ \%$

Flask 8

Mean = $0.326\underline{+0.324+0.332+0.153}{4} = 0.284$

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.326-0.284)^2 + (0.324-0.284)^2 + (0.332-0.284)^2 + (0.284-0.153)^2$$

$$= 0.0755$$

%CV= $\sigma / \mu * 100\% = 0.075/0.284 * 100 = 26.41\%$

Flask 9

Mean = $0.354\underline{+0.264+0.355+0.279}{4} = 0.313$

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.354-0.313)^2 + (0.313-0.264)^2 + (0.355-0.313)^2 + (0.313-0.279)^2$$

= 0.0418

%CV= σ /μ * 100% = 0.0418/0.313 * 100 = 13.35%

Flask 10

Mean = 0.411+0.119+0.474+0.197 = 0.300
4

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}}$$ = Sqrt $(0.411-0.300)^2 + (0.300-0.119)^2 + (0.474-0.300)^2 + (0.300-0.197)^2$

= 0.147

%CV= σ /μ * 100% = 0.147/0.300 * 100 = 49.00%

Flask 11

Mean = 0.427+0.062+0.390+0.048 = 0.232
4

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}}$$ = Sqrt $(0.427-0.232)^2 + (0.232-0.062)^2 + (0.390-0.232)^2 + (0.232-0.048)^2$

= 0.177

%CV= σ /μ * 100% = 0.177/0.232 * 100 = 76.29 %

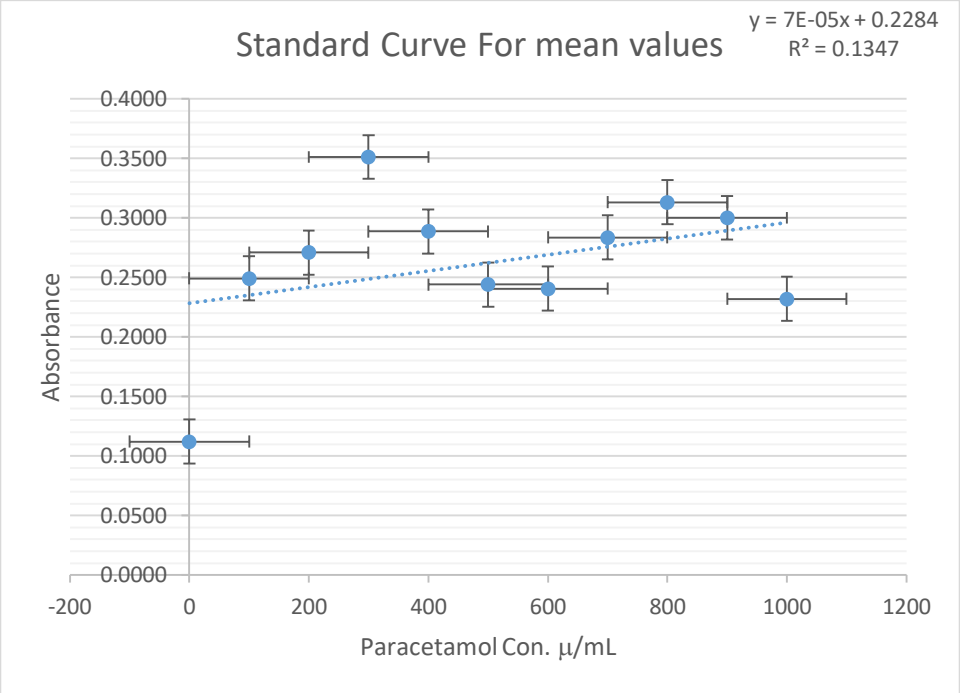
Flask 12

Mean = 0.195+0.040+0.249+0.044 = 0.132
4

$$\sigma = \sigma_X = \sqrt{\frac{1}{n}\left\{\sum_{i=1}^{n} X_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} X_i\right)^2\right\}} = \text{Sqrt } (0.195\text{-}0.132)^2 + (0.132\text{-}0.040)^2 + (0.249\text{-}0.132)^2 + (0.132\text{-}0.044)^2$$

$$= 0.082$$

%CV= σ /μ * 100% = 0.082/0.132 * 100 = 62.12 %



Calculating the concentration for the unknown

Absorbance of the Unknown = Y = 0.132= 0.00007x + 0.2284

0.00007x = 0.132-0.2284

0.00007x = 1377

X = 0.0964/0.00007 = 1377

Therefore the concentration of the unknown is 1377 μg/mL

Discussion

This experiment's scope was to precisely and quantitatively ascertain the paracetamol concentration by way of the colourimetric assay. The first step in this practice was to make standard solutions of paracetamol of different concentrations which were then reacted with nitrous acid (Shihana et al., 2010). The unknown concentration of the paracetamol was found to 1377 μg/mL. However, I feel that this experiment had errors, especially when running the UV analysis. Most of the replicates readings were significantly different, and this had an impact on the final results. The errors could have been due to poor mixing of solutions or oversight in reading volumes

Conclusions

The paracetamol tablet's colourimetric assay followed the conventional methodology where statistical testing was conducted on the results, and a standard curve was plotted. However, from the regression analysis of the curve, one can tell that there must have been some errors in the UV data generated. It could have been attributed to both errors of omission and commission.

References

Shihana, F., Dissanayake, D., Dargan, P. and Dawson, A., 2010. A modified low-cost colorimetric method for paracetamol (acetaminophen) measurement in plasma. *Clinical toxicology*, *48*(1), pp.42-46.

Wilkinson, G.S., 1976. Rapid determination of plasma paracetamol. *Annals of clinical biochemistry*, *13*(1-6), pp.435-437.

Artemia Salina Bioassay

Introduction

Artemia salina is a type of a shrimp and belongs to the class *halophilic crustacean*. Artemia can be found in salty marshy places. It is believed that *Artemia salina* used to previously live the oceans but migrated to safer salt marsh setting free from predators. As a result of its ability to generate dormant cysts eggs, Artemia has been used as test kits of toxicants. This kit provides a ready supply of biological material that can be stored for longer time (often more than eight months) without losing their viability and without the need of continuously keeping culture cells for tests (Caldwell, Bentley and Olive, 2003). Artemia salina hatch by adding their eggs to salty water at least 48 hours before commencing the test with consequent incubation at room temperature in. This report gives the outcome of the bioassay of *Artemia salina* in terms of mortality rates and dose standard curves L_{50}.

Materials and Methods

During the first day, 10 mls of 0, 10, 18, 32, 56 and 100 ppm of the potassium dichromate stock solution was made using seawater as the solvent. The formula used to prepare the concentration include is $C_1V_1=C_2V_2$. Consequently, 2 mls of seawater was added to each of the wells in columns1 to 6 of the 24-well plate provided. Column 1 is the control column. Precisely 2 mls of the chemical concentrations prepared was added to columns 2 through 6. In the following manner

Column 2 = 10 ppm

Column 3 = 18 ppm

Column 4 = 32 ppm

Column 5 = 56 ppm

Column 6 = 100 ppm

The *Artemia salina* was then transferred to the test wells in two phases. For the first phase, about 50 larvae were transferred from the petri dish to the rinsing wells. This step was meant to minimise the concentration of the toxicant solution contained in the treatment wells. For the second phase, 10 larvae from the rinsing wells were transferred to each of the treatment wells bearing in mind that the concentrations were performed in triplicates. It is important to note that the number of larvae deposited in each well had to be recorded. The larvae were counted as they exited the tip of the pipette. The lid on the 24-well was replaced and incubated in the dark at room temperature for 24 hours.

During the second day, after the incubation time had elapsed, the 24-well plate was investigated under the microscope where the number of dead larvae in each well was counted. The number of lifeless larvae in each of the well was subtracted from the total number of larvae deposited into each well on the first day, which presents the number of alive larvae in every well.

Results

The formula for calculating the mortality of *Artemia salina* is given as;

$$\% \text{ Mortality} = 1 - \frac{\%\text{Treatment (Live)}}{\%\text{ Control (Live)}} \times 100$$

Where:	Treatment concentrations.	=	No. of larvae alive in the wells with chemical
	Control	=	No. of larvae alive in wells with just diluent
	X 100	=	to get percentage

During the experiment, 10 *Artemia salina* larvae were deposited into each well of the 24-well plate on the first day. At the end of the incubation period, the number of moribund larvae in each well was documented. The number of larvae that were still alive was computed in terms of mortality rate.

Number of alive larvae

	Conc.	0 (ppm)	10 (ppm)	18 (ppm)	32 (ppm)	56 (ppm)	100 (ppm)
Number	A	10	8	5	3	2	0
Alive	B	9	7	6	4	3	0
	C	9	9	5	4	1	0
	Total Alive	28	24	16	11	6	0

Calculation of the mortality rate

Using 0 ppm concentration

Mortality rate = 1-28/28 X 100%

Mortality rate = (1-1) x 100%

Mortality rate = 0%

Using 10 ppm concentration

Mortality rate = 1-24/28 X 100%

Mortality rate = (1-0.857) x 100%

Mortality rate = 14.30 %

Using 18 ppm concentration

Mortality rate = 1-16/28 X 100%

Mortality rate = (1- 0.571) x 100%

Mortality rate = 42.90 %

Using 32 ppm concentration

Mortality rate = 1-11/28 X 100%

Mortality rate = (1-0.393) x 100%

Mortality rate = 60.70 %

Using 56ppm concentration

Mortality rate = 1-6/28 X 100%

Mortality rate = (1-0.214) x 100%

Mortality rate = 78.60 %

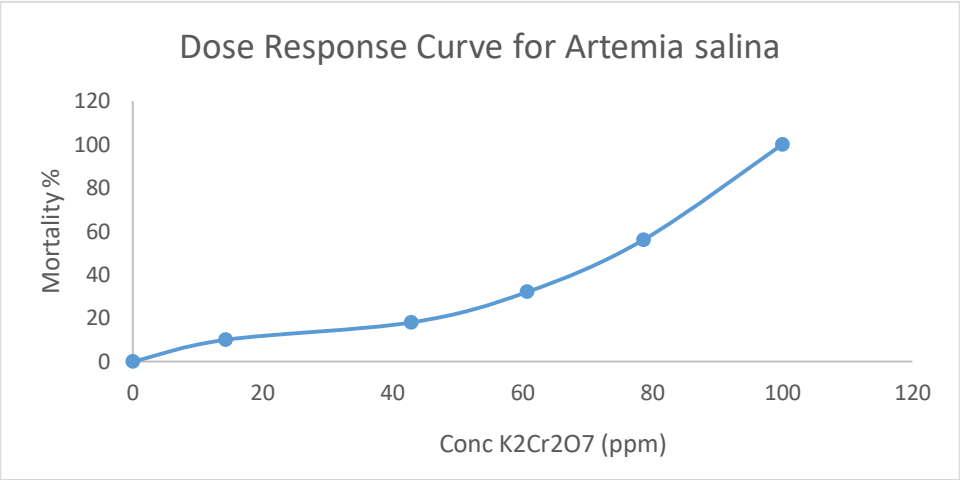
Using 100 ppm concentration

Mortality rate = 1-0/28 X 100%

Mortality rate = (1-0) x 100%

Mortality rate = 100.00 %

Dose-response Curve



From the curve, the LC_{50} (24hrs) can be determined as about 75 PPM.

Discussion

These experimental results show that the mortality rate of Artemia salina increases with the increasing concentration of potassium dichromate (Ferraz Filha *et al.*, 2012). From the dose curve, the L_{50} of the larvae seem to be about 75 ppm.

Conclusion

It can be concluded that Artemia appears to be more active at moderate concentrations of potassium dichromate.

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The Compound Microscope

Introduction

Factually speaking, microbiology deals with living organisms that are almost infinitesimal and cannot be viewed with the naked eye. Microbiology owes its origin to the invention of the microscope. The first light microscope was simple in design, having a single lens system. However, modern light microscopes have evolved and are now referred to as compound microscopes as they have two lens systems that enable them to have a highly potent magnifying strength (Hayley anderson, 2010; Qu, Wang, Cheng, & Asundi, 2013; Takajo, Okawa, Hasegawa, & Aono, 2007). Thus an in-depth knowledge of the individual elements of a compound microscope and their application is imperative for every microbiologist.

Objectives of the experiment.

To familiarise oneself with;

- ✓ Theoretical concepts of the bright field microscopy.
- ✓ The parts of a compound microscope.
- ✓ How to use and maintain a compound microscope.
- ✓ Practical application and maintenance of the compound microscope for visualisation of cell structure using stained slides

Materials and Methods

A compound microscope was on the laboratory bench where a lens paper was used to clean the ocular, objective and condenser lens. After that, the light source was turned on, and

the microscope was fitted with an adjustable condenser, then elevated to the level of the stage. The microscope's nosepiece was rotated nosepiece to bring the low power objective (10x) into the correct position. After that, the specimen slide was placed on the stage and placed in the centre so that it was illuminated. Using the coarse adjustment knob, the 10x objective was raised upward to its stop position. This process was repeated with the fine focus knob. While looking at the slide, the objective was slowly lowered with the coarse focus knob until it came into focus (Frost, Wang, & Brandon, 2007). The coarse focus was left untouched since compound microscopes are a parfocal system. The fine focus adjustment was used to bring the specimen into clear focus. In the meantime, the iris diaphragm was adjusted to achieve an optimal contrast between the specimen and the background. After that, a small immersion of oil was placed on the slide, and oil immersion lens was swung into position. Consequently, all the observations were recorded.

Results and Discussion

The laboratory experiment began with an exploration of the history, critical parts of a microscope, and microscope applications. The microscope is a laboratory tool invented by Anton Von Leeuwenhoek and later upgraded by the Janssen brothers. It was used to enlarge the view of things that could not be visualised by the naked eye, and that is the primary reason why a microscope is important in the study of life.

Basic understanding of optics and contrasting techniques is critical when using microscopic imaging. The accurate setup of a compound microscope including correct illumination enhances the image clarity, and this is the foundation for an optimised image analysis. A compound microscope can distinguish between stained and unstained specimens

affecting the amplitude and the phase of the light waves passing through the specimen (Simon & Comastri, 2005). With the technological revolution and improved optics, the modern microscope can be an image as small 200nm in diameter.

Magnification is how many times an object is optimised, represented by the letter "x". A microscope can magnify images to up to 500,000 x depending on the kind of the microscope is being utilised. Total magnification is often calculated by multiplying the ocular magnification by magnifying the objective lensed being used. For example, in this experiment, we worked with a compound microscope that provides maximum magnification and optimised resolution.

The objective lens is usually closer to the sample. It can magnify it and generate an actual image projected to the focal plane and subsequently enlarged by the ocular lens to generate the final vie of the amplified image. According to Frost et al., (2007), the most commonly utilised microscopes have a revolving nosepiece that accommodates between three and four objective lenses with magnification factors of 4x, 10x, 40x and 100x each possessing a different degree of magnification. The magnifying power of a lens is the inverse proportion to its focal lengths and the objectives' focal lengths. In microbiological work are 16mm, 4mm and 1.8mm which magnify approx. 10, 40 and 100 times, respectively. Accordingly, combining this with the ocular lens magnification results in the total magnification of the sample. The total magnification for the scanner, LPO and HPO is 40x, 100x, and 400x respectively. The total magnification is computed by multiplying the ocular's magnification which is 10x with the objectives respective magnification which is 4x, 10x and 40x. As we move on with our experiment, we moved the slide to the right using the x-axis knob. We noticed that as we move the slide to the right direction, the ocular image moved in a directly opposite direction. After we tried to move the slide to the left and the same thing happened; it moved oppositely. Lastly, we

tried to move the slide forward and backwards using the y-axis knob and just like what happened in the x-axis, it also moved in the opposite direction.

Conclusion

The objective of the experiment was achieved. It can be concluded that the study shed light on the theoretical framework of the light microscopy. I learned about the different components of a compound microscope, how to use and maintain it.

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The Gram Stain

Introduction

One of the commonly used procedures for identifying and differentiating different kinds of bacteria is gram stain techniques. Generally speaking, most bacteria can be classified into two groups, depending on whether they keep or lose their main stain (crystal violet). The bacteria which keep the crystal violet stain classified as gram-positive while those that lose their crystal violet stain and consequently stained by safranin or methyl red are categorised as gram-positive bacteria (Beveridge, 2001). Gram staining can be viewed as a differential staining method since it can distinguish the two bacterial groups from one another.

Materials and Methods

Materials:

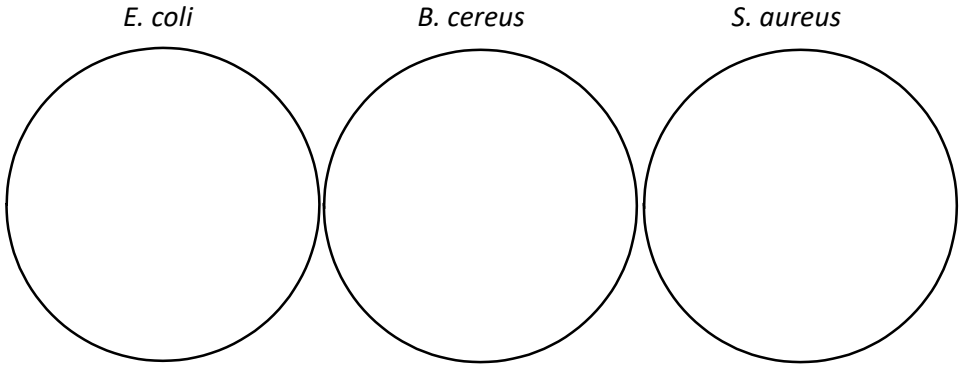
The required materials included pure cultures of Bacillus cereus, Staphylococcus aureus and Escherichia coli, the staining solutions crystal violet, Grams iodine, 95% ethyl alcohol and safranin.

Method

The individual smears of Bacillus cereus, Staphylococcus aureus and Escherichia coli were prepared. On another slide, a smear of Staphylococcus aureus and Escherichia coli was prepared and fixed. The smears were stained with crystal violet for 1 minute, then washed with water for a few seconds. After that, excess water was drained off. Consequently, grams iodine was applied for 1 minute, then washed with water and drained off. While holding the slide at a 45^{o} angle, the smears were decolourised with 95% ethyl alcohol until

the free colour has been washed off. The slides were then washed the water drained off. The smears were counterstained for 30 seconds to 1 minute with safranin, and then washed with water and blotted dry (Moyes, Reynolds, & Breakwell, 2009). A microscopic examination of each stained preparation was made using x10 objective lens.

Results



Cell morphology:

Shape	Bacilli	Bacilli	Cocci
Arrangement	Streptobacillus	Diplobacillus	Staphylococcus
Cell colour	Red	Purple	Purple
Gram reaction	Negative	Positive	Positive

Discussion

Based on the data obtained from the experiment, it can be deduced that the presented results did, in fact, support our hypothesis that *Escherichia coli* is a gram-negative bacteria by virtue of the fact that it had red colour wile Bacillus cereus and Staphylococcus aureus are gram-

positive bacteria given that they had a purple stain. There is no contradiction between the findings of this study and those of previous studies (Beveridge, 2001; Moyes et al., 2009).

Conclusion

To sum up, Gram staining comprises one of the most fundamental types of differential staining. It can classify bacteria into two major groups, namely; gram-positive and gram-negative bacteria. Gram-positive bacteria do not lose the colour of the main stain, which is purple, and on the other hand, gram-negative bacteria have the colour obtains the red colour as a result of the counterstain. Consequently, Gram-positive bacteria are endowed with a thicker cell wall, which mainly comprises peptidoglycan. This is believed to be why it was able to keep the purple colour. On the contrary, the gram-negative bacteria cannot retain the purple colour since it is made up of a thinner cell wall. In the lab experiment, it was concluded that *B. cereus and S. aureus* were both gram-positive bacteria while *E.Coli* was gram-negative bacterial.

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Streaking For Isolation

Introduction

In the 1800s, a scientist called Loius Pasteur came up with the concept of pure culture. This was seen as a critical intellectual step in the right directions since the capacity to work with pure cultures enabled Pasteur and other microbiologists to examine discrete bacterial species. Pasteur's technique for processing pure cultures by constantly diluting broth cultures was very effective. During the 1880s, Robert Koch invented the method of streaking for isolation. The the goal of this technique was to come up with isolated colonies. An isolated colony comprises numerous cells identical in nature and are progenies of a single author cell (Abdulkadir & Waliyu, 2012; Eyler, 2013). Koch was able to select cells from a single colony to inject another culture. This method provided a powerful new strategy for creating pure cultures. Consequently, streaking for isolation has now be perceived as a standard protocol in every microbiology laboratory. The bottom line in streaking technique is that one has to spread the cells on an agar surface in the quest to ensure that individual cells can develop

Materials and Methods

Materials

Agar culture of Staphylococcus aureus and E.Coli demonstrating isolated colonies

Method

The bottom of the agar plate's perimeter was properly labelled using initials, date, medium, inoculum, incubation temperature, and agar type. After that, a small amount of a well-isolated colony from the stock culture was transferred to, transferred to Sector1 of the agar plate using the Aseptic Technique. As a reminder, the loop was cooled each time after it was flamed. **The**

process was started from one end of the sector and then proceeded to move in a zigzag direction as illustrated by the lecturer. The plate was then rotated at an angle of 90 degrees were the cells where spread from sector 1 into sector 2 while sterilising the loop. This was done to ensure that all the cells on the loop destroyed. It is important because since the objective was to spread out the cells from sector 1. The loop was moved the back and forth from sector 1 to sector 2 about five times, and after that, sector 2 was inoculated in the same zigzag manner as was done in sector 1. This step was repeated to spread cells from sector 2 to sector 3.

The plates were inverted and then incubated at a relevant temperature.

Results

We took our petri dish and marked the bottom of it. Then we streaked a loopful of bacteria. After that, we burn the loop and cooled it twice and then streaked the second and last section. The outcome was as follows;

Discussion

The primary objective of the streak technique is to generate separated colonies of bacteria from cells concentrates. To attain this objective, the bacterial stock had to be streaked with an

inoculating loop into a solid medium in steps. Accordingly, each streaking step was succeeded by a flaming phase. As bacteria cells are streaked from one point of
the plate to the other, a dilution occurs. During the final streak, the number of bacterial cells on the loop ought to be small. As these small quantities of cells are distributed across the remaining parts of the plate, they end up being physically isolated, and each isolated cell will be divided. As the dividing cells continue to flock in the initially isolated cell's local area, a discrete cluster of cells becomes noticeable. This cluster of cells is what is referred to as a colony (Abdulkadir & Waliyu, 2012)**.** The entire cells comprise the offspring of an individual cell from the initial sample.

It has to be noted that the separated colonies of various kinds of bacteria will portray diverse cell morphologies. Furthermore, culture purity can be ascertained through observing the morphology of separated colonies. A streak plate consisting of mixed culture will portray the colony types of individual cultures. To separate pure cultures of individual cell cultures existing in a mixed culture, one particular colony can be selected, freshly restreaked and then incubated (Eyler, 2013). Observation of similar colonies on this second plate will indicate a pure culture. Most microbiologists repeat streaking from an isolated colony two to three times before they guarantee culture purity.

The utilisation of the streak plate method needs knowledge of rules of aseptic applications. When working with a bacteria culture, a microbiologist needs to proceed with care to ensure that the culture is not contaminated during the manipulations, and with caution that no culture escapes into the environment. Also, all culture vessels and laboratory media must be free from bacteria - sterile. Sterilisation procedures include treatment with high temperature and

irradiation. Some culture media are filtered to remove all bacteria. Inoculating loops are exposed to a flame for sterilisation.

When working with a mixed culture, the streak plate is used to isolated colonies of each culture in the mix. Once isolated, each colony can be restreaked to prepare pure cultures. To maintain a pure culture, there are several options. Many cultures will survive in the refrigerator for short periods. The time will vary from weeks to months (some will last years). Refrigeration is thus used for short term storage of cultures. Permanent storage uses the -80C freezers, and for cultures regularly used in the lab, one may safely keep the culture by combining refrigeration and bacterial subculture.

Conclusion

Streaking is important because the human body has billions of bacteria which constitutes the normal flora fighting against invading pathogens. Streak plate technique is used to grow bacteria on a growth media surface so that individual bacterial colonies are isolated and sampled.

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Quantitative Determination of Total Coliforms and E.coli in Water by Estimating the Most Probable Number (MPN) and Membrane Filtration.

Introduction

This exercise demonstrates the use of selective media in the quantitative determination of water quality indicator bacteria by using a serial dilution and statistical calculation of the most probable number that would give the observed results. This is generally referred to as MPN. The MPN is performed by inoculating replicates of decimal dilutions of the sample into liquid media (Gronewold & Wolpert, 2008; Ramteke, 1995; Schulz, Peréz-de-Mora, Engel, Munch, & Schloter, 2010). Bacterial densities are estimated by a combination of positive (growth and/or fermentative gas production), and negative results read from the MPN table. This procedure consists of two stages,

(a) – The **presumptive test** consists of reading the tubes for positive and negative results to obtain a preliminary estimate of bacterial density.

(b) The **confirmed test** consists of inoculating aliquots of the presumptive test's positive tubes onto a more selective medium.

MPN is a statistical enumeration of specific organisms. Its precision and reliability are practically determined by the number of replicate tubes used for each dilution examined. Since the MPN tables are based on probability calculations, an inherent 23% bias in the three dilution series is normally used. Other disadvantages of the procedure include the time require (as long as 96 hours for result), the amount of glassware required and the inability to lend itself to fieldwork. Advantages of the procedure include minimal training/experience for the analyst, high turbidity

or algal concentrations have no apparent effect on the results. This method may be the only applicable method for analysing materials such as muds, soils, sediments and bottom sludges.

Materials and Methods

Part 1

Precisely, 10ml of the aquatic sample was transferred in triplicate into 10ml of double strength MacConkey broth. After that, 1ml of the aquatic sample was transferred in triplicate into 5ml of single strength MacConkey broth. Accordingly, 0.1ml of the aquatic sample was transferred in triplicate ito 5ml of single strength MacConkey broth (Dehority, Tirabasso, & Grifo, 1989). After that, it was 37^{o}C for 24hours. Tubes showing acid and gas production after 24hours should be regarded as presumptive positives, and confirmatory tests should be carried out on these.

Part 2

MPN Confirmatory Test

E.coli

Subculture a loop full of the positive tubes to Brilliant Green Bile Lactose Broth (BGBLB), and Tryptone broth Incubate at 44^{o}C for 24hours. Growth in the BGBLB at 44^{o}C and a positive Indole test is confirmation of the presence of E.coli. A positive Indole test is the presence of a red ring after adding 0.2 -0.3 ml Kovac's reagent.

Total Coliforms

Subculture a loop full of the positive tubes to Brilliant Green Bile Lactose Broth (BGBLB). Incubate at 37ºC for 24hours. Growth in the BGBLB at 37ºC is confirmation of the presence of total coliforms.

Results

RESULTS

Table1. The Most Probable Number (MPN) of coliform in three media incubated at 37 ℃ for 24 hours.

Product	LST MPN/g	BGLB MPN/g	EC MPN/g	Typical Colonies of EMB
Water 160	+		+	Yes

*Prepared by stomaching

EMB – Bright green colonies E.Coli conformed
Gram stain –pink rod-shaped –negative

Table 2: Results of IMViC tests

Indole-reddish pink +

Voges-Proskauer (VP)-cloudy brown ppt Methyl red-reactive compounds-bright yellow

Citrate -clear

Medium	10 -1	10-2	10-3
LST	1	3	1
BGLB	1	3	1
EC BROTH	1	1	1

LST = based on the table =1,and 2 =20
MPN from table /100 * dilution factor from middle tube = MPN/G

20/100*1000= 200g

BGLB= 1,3,1= 20 =200g

EC BROTH= 1,1,1=11

11/100*1000 =110g

Discussion

Brilliant Green Lactose Bile Broth (BGLB) has a positive result after incubation at 35℃ for 24 hours because inside the tubes, the broth is turbid and all of them produce gas. Simultaneously, the transferred loop from LST to EC broth has completed incubation at 45℃ for 24 hours, and the result is also positive due to gas production. For EMB plate, the streaks are flat colonies with metallic green sheen after 24 hours at 37℃ incubation. Moreover, for IMViC tests, both Indole production Is positive, whereas the others are negative.

Conclusion

To get rid of *E.coli,* water should be properly boiled 100℃ so that it can be destroyed. However, *E.coli* is found in other foods and liquids, so sanitation is extremely significant and avoid cross-contamination. For people who sell drinking water, it is best to keep the water refrigerated.

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Membrane Filter (MF) Technique

Introduction

The membrane filter also referred to as technique (MF) can be utilised to give a fairly accurate direct count of cell colonies present on the surface of a filter. It has been thought that the MF technique is more advantageous than MPN due to the short period of incubation. Thus larger sample aliquots can be effectively tested, therefore increasing the integrity of the study found. Other benefits of using MF include optimised precision, less time used and its versatility applications in research. However, according to Jacobs, Zeigler, Reed, Stukel, & Rice, (1986), the limitations of MF arise during the composition of the samples due to problems with working with membrane filters. Furthermore, MF may not be useful for samples that have high turbidity and small bacteria counts. Besides, large non-specific samples may disguise the actual appearance on indicators, and also toxic substances may obstruct the indicators from manifesting.

Materials and Methods

Materials

Filtered- River water samples (undiluted) 10.0, 25.0, and 50.0 ml.

Method

Utilising the aseptic method, a sterilised filter was placed into the filtering membranes, and the funnel was then fixed to the flask. The sample in the container was the vehemently shaken before filtration. After that, the indicated volume was pipetted into the funnel, and a vacuum was subsequently applied to filter the sample. To prevent the sample from being sucked and lost by

backpressure, the aspirator hose was removed from the pump first, and water was turned off. The sterilised absorbent pads were placed one the sterile Petri dishes where 2.0 – 3.0 ml of membrane enriched teepol broth was added to each of the plates. The excess liquid was discarded immediately before use to prevent concurrent development on the membrane. The funnel has then removed the funnel and filter were aseptically positioned upwards on the teepol broth absorbent pads. Consequently, two plates were incubated at 37ºC for 24 hours and the other at 44ºC for 24 hours. The existence of yellow colonies on the 37ºC plates was meant to indicate coliforms, whereas the yellow colonies on the 44ºC plates were supposed to be reflective of E.coli.

MF Confirmatory test

Two yellow colonies were streaked from the teepol broth into Mendo agar and afterwards incubated at 37º C for 24 hours. Generally speaking, coliform colonies manifest themselves as red colonies with a green metallic sheen.

Results

Colour and morphology of all colonies formed.

Media	**m-ENDO**		
volume	**10ml**	**25ml**	**50ml**
No of colonies	1	1	1
Appearance	Media changed colour from pink to rose-red, Green metallic sheen, Large, circular colonies	The Media changed colour from pink to red, Green metallic sheen, Large, circular colonies	Media changed colour from pink to rose-red, Green metallic sheen, Large, circular colonies
Negative control	No growth	No growth	No growth

Confirmation of the presence of E.Coli in m-Endo medium using teepol broth incubated at 37^{o}C and 44^{o}C for 24 hrs.

Tubes	**Plate 1**	**Plate 2**
Appearance	Turbid growth	Turbid growth
Gas production	Gas formation	Gas formation

Discussion

The coliform colonies' appearance did not come as a surprise and it was the colour changed in the medium from pink to red, and there were manifest large circular colonies on green metallic sheen. The m-Endo agars is used for isolating and quantifying E.Coli in water via MF. According to Sandhya, Uma, & Subbarao (1999), turbidity alone does not reflect a positive confirmation for coliforms' presence. The turbidity also has to be accompanied by production of gas. Based on the results, a turbid growth and generation of gas in plates 1 and 2 observed, and this was indicative of the presence of E.Coli. The finding of this study is in agreement with studies done by others.

Conclusion

The MF test was successful and took less time than MPN. The study has successfully used the MF technique to prove the presence and identify colonies and E.coli in river water.

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