



FORMULATION, PHYSICOCHEMICAL CHARACTERIZATION AND STANDARDIZATION OF TRIS BUFFER SOLUTION PREPARED FROM LOCALLY AVAILABLE REAGENTS

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ABSTRACT: tris buffer is a solution made primarily from tris(hydroxymethyl)aminomethane (Tris) and an acid, commonly hydrochloric acid (HCl), to maintain a relatively constant pH when small amounts of acid or base are added. Tris buffer solution was prepared using local raw materials by dissolving 5g of boric acid, 6g of EDTA, and 50g of trisma base 8n 1000cm³ of distilled water. Comparative analysis was done on the local produce tris-buffer solution against commercial tris-buffer solution produced using pH meter for the pH. The result obtained showed that Tris-buffer solution locally produced with local materials has colour changes range of the acid-base indicators with changing in temperature while its minimum pH was 7 while the maximum pH was 12 and contained appropriate amount of chemical compositions with a good of average titration values. The study concluded that tris-buffer solution prepared from local materials is suitable for scientific experiments. It was also concluded that the colorimetric acid-base titration used to calibrate the HCl to the Tris-buffer solution allowed for significant saving on the local materials used for tris-buffer prepared. The study recommends that this locally produced from local raw materials can be used in the laboratories especially institutions' laboratories for the scientific experiments instead of using commercial tris-buffer solution which is costly and its materials not readily available unlike local tris-buffer solution.

Keywords: Tris-buffer solution, pH, Titration, Acid-base, Calibrate, HCl, EDTA

1.0 INTRODUCTION

Tris-buffer solutions can be defined as water-based liquids that include both a weak acid and its conjugate base (Anthony, 2018). Because of its chemistry, buffer solutions can keep pH (acidity) at a nearly-constant level even when chemical changes are taking place. Buffer systems occur in nature, but they are also extremely useful in chemistry. Almost all chemical, biochemical, biological processes are temperature, ionic concentration and pH dependent. Even a slight change in temperature, ionic concentration and pH can result in metabolic acidosis or alkalosis, resulting in severe metabolic complications. The purpose of a buffer in a chemical, biochemical, biological system is to maintain intracellular and extracellular temperature, ionic concentration and pH within a very narrow range and resist changes in temperature and pH in the presence of internal and external influences (Chandra, 2019). Buffer solutions are water-based liquids that include both a weak acid and its conjugate base. Because of their chemistry, buffer solutions can keep pH (acidity) at a nearly-constant level even when chemical changes are taking place. Buffer systems occur in nature, but they are also extremely useful in chemistry (Chandra, 2019).

Buffers solution are aqueous systems that resist changes in temperature, ionic concentration and pH dependent when small amounts of acid or base are added. Buffer solutions are composed of a weak acid (the proton donor) and its conjugate base (the proton acceptor). Buffering results from two reversible reaction equilibria in a solution

wherein the concentration of proton donor and its conjugate proton acceptor are equal. For example, in a buffer system when the concentration of acetic acid and acetate ions are equal, addition of small amounts of acid or base do not have any detectable influence on the temperature, ionic concentration and pH. This point is commonly known as the isoelectric point. At this point there is no net charge and pH at this point is equal to pKa (Mohan, 2021).

According to Anthony (2018), tris-buffers have both intensive and extensive properties. The intensive property is a function of the pKa value of the tris-buffer acid or base. Most simple buffers work effectively in the pH scale of $pK_a \pm 1.0$. The extensive property of the tris-buffers is also known as the buffer capacity. It is a measure of the protection a tris-buffer offers against changes in temperature, ionic concentration and pH. Tris-buffer capacity generally depends on the concentration of buffer solution. Tris-buffers with higher concentrations offer higher buffering capacity and this solution is a commonly used buffer solution in biochemical and molecular biology experiments. It is a good choice for maintaining a stable pH under various conditions (Anthony, 2018). On the other hand, temperature, ionic concentration and pH are dependent not on the absolute concentrations of tris-buffer components, but on their ratio. Tris-buffer capacity is a term used to describe the ability of a given buffer to resist changes in temperature, ionic concentration and pH on addition of acid or base. A tris-buffer capacity of 1 is when 1 mol of acid or alkali is added to 1 liter of buffer and pH changes by 1 unit. The tris-buffer capacity of a mixed weak acid-base buffer is much greater when the individual pKa values are in close proximity with each other. It is important to note that the tris-buffer capacity of a mixture of buffers is additive. Using the above equation, known that when $pH = pK_a$ the concentrations of acetic acid and acetate ion are equal. Using a hypothetical buffer system of HA ($pK_a = 7.0$) and $[A^-]$, tris-buffer solution is a solution demonstrate how the hydrogen ion concentration, $[H^+]$, is relatively insensitive to external influence because of the buffering action (Anthony, 2018). For example: If 100 ml of 10 mM (1×10^{-2} M) HCl are added to 1.0 liter of 1.0 M NaCl at pH 7.0, the hydrogen ion concentration, $[H^+]$, of the resulting 1.1 liter of solution (Anthony, 2018)

Tris-buffer, also known as tris (hydroxymethyl) aminomethane tris-buffer, is a widely used chemical tris-buffer in biochemistry, molecular biology, and clinical laboratories (Maity *et al.*, 2024). It plays a vital role in maintaining a stable temperature, ionic concentration and pH environment, which is essential for many biological processes, including enzyme activity, nucleic acid reactions, and protein handling. Due to its chemical properties. Tris is highly versatile and effective in regulating temperature, ionic concentration and pH within the physiological range of pH 7.0 to 9.0, making it suitable for tris-buffers used in experiments such as electrophoresis, Western blotting, and cell culture (Kuril *et al.*, 2024).

Martin et al (2019) described Tris-buffer as a solution that has the ability to resist change in temperature and pH, when small amounts of strong acids or bases are added to it. For example, when 0.01 mole of strong acids or base is added to distilled water, the temperature, ionic concentration and pH drops to 2 with the acids and rises to 12 with the base. If the same amount of acid or base is added to an acetic acid-sodium acetate buffer, the temperature, ionic concentration and pH may only change a fraction of a unit (Martin et al., 2019).

Tris-buffers are important in many areas of chemistry. When the pH must be controlled during the course of a reaction, the solutions are often buffered. This is often the case in biochemistry when enzymes or proteins are being studied. Our blood is often buffered to a pH of 7.4. Variations of a few tenths of a pH unit can cause illness or death. Acidosis is the condition when pH drops too low. Alkalosis is the condition when pH becomes higher than normal. Acidosis or alkalosis affects the functions of the heart (Anthony, 2018).

In Tris-buffer solution, two species are required one is capable of reaction with OH^- and the other will react with H_3O^+ . The two species must not react with each other. Many tris-buffers are prepared by combining a weak acid and its conjugate (acetic acid and sodium acetate) or a weak base and its conjugate (ammonia and ammonium chloride). In general, the pH range in which a tris-buffer solution is effective is + or – one pH unit on either side of the pka. The need for a Tris-tris-buffer arises because many biochemical reactions are pH-sensitive (Gonzalez-Posada *et al.*, 2024), and even minor fluctuations can impair enzyme functions or alter molecular structures. Tris tris-buffers are known for their temperature, ionic concentration and pH stability (Chheda *et al.*, 2024), especially in solutions subjected to varying conditions, such as changes in temperature or ionic concentration. Hence, the present study aimed to carry out the study on characterization and standardization of Tris-buffer solution produced from local raw materials (Anthony, 2018).

Although Tris-buffer solutions are indispensable in scientific research (Wang *et al.*, 2024), their production and availability depend heavily on importation, especially in developing countries, resulting in high costs and supply-chain challenges. This importation has provided a great limitation to the nation science and technology

advancement. This also creates a bottleneck for laboratories that operate with limited resources. Local production of Tris-buffer using accessible raw materials offers a potential solution to reduce costs and promote sustainable practices (Gorjanović *et al.*, 2024).

This study aims to explore the feasibility of producing high-quality Tris-buffer solutions from locally available raw materials. It seeks to characterize and standardize the buffer to ensure it meets laboratory-grade specifications. By developing a local alternative, the study hopes to reduce reliance on imported chemical reagents, lower production costs, and enhance the accessibility of essential laboratory materials for research institutions. The aim of this study is to characterize and standardize Tris-buffer solution produced from local raw materials.

2.0 METHODOLOGY

2.1 MATERIALS

2.1.1 Sample

The samples used for the study are 100mL of tris(hydroxymethyl) aminomethane, deionized water, 121.14g/mole of TRIS, glycine, phenol and acetic acid.

2.1.2 Reagents

The reagent includes Distilled water, MgCl₂, CaCl₂, methyl red indicator, HCl, Na₂SO₄, KCl, AgCl, AgNO₃ Formaldehyde, Ethanol, Ammonia, hydrochloric acid (HCl), Sodium Hydroxide, and Sodium Hydroxide.

2.1.3 Apparatus

The apparatus used for study include Beakers, Water bath, Round bottom flask, samples’ bottle, aluminum foil, retort stand, puppet, weighing apparatus, pH meter, Calibrator, pH meter and mixer.

2.1.4 Sample Collection

The local materials used for the study were collected from chemical industry fertilizer plants, coolants, soap making, sugarcane plant in Osun State Water Corporation and textile industry at Alekuwode Area, Osogbo of Osun State.

Raw Material	Role	Local Sources
Formaldehyde	Carbon backbone	Chemical Shop at Ede
Ammonia	Nitrogen source	Fertilizer plants, industrial coolants
Sodium Hydroxide	pH adjustment	Soap-making, textile industries
Ethanol	Recrystallization solvent	Distilleries, sugarcane plants
Distilled Water	Reaction solvent	Water treatment plants, labs (Water Corporation, Ede)

2.1.5 Method of Preparation

➤ Ratio Calculation

The required amount of Tris and hydrochloric acid based was calculated based on the required buffer concentration and pH value. The concentration (molarity) and volume of Tris buffer that the study application calls for were determined. Also, the number of moles of Tris that are required was determined by multiplying the molar concentration of buffer by the volume of the buffer that is being made.

Moles of Tris = $\frac{\text{mol}}{(\text{L} \times \text{L})}$ (i)

The grams of solid Tris were determined by multiplying the number of moles by the molecular weight of Tris (121.14 g/mol).

Grams of Tris = (moles) x (121.14 g/mol)(ii)

➤ Simplifying Buffer Preparation

The TRIS buffer solutions were prepared using highly purify and carefully characterized reagents with the use of doubly distilled and coulometrically standardized HCl using TRIS from Science Laboratory Technology Department Laboratory of certified purity and salt that had been purified by recrystallization (i.e. dissolved in deionized water followed by precipitation by partially evaporated the solution). The HCl, Na₂SO₄ and KCl were dried thoroughly by recrystallization. The recrystallized MgCl₂ and CaCl₂ were prepared into a solution. The two solutions were subsequently calibrated by analyzed their chloride contents through precipitation of AgCl and AgNO₃ was added in excess. All buffer components were weighed (W₁) and added deionized water to the solution and reweighed again (W₂)

➤ Preparation of Tris-Buffer Solution

Primary Buffer Solution: NaCl, CaCl₂ and KCl salt solution were dried at 200°C for at least 4 hours and cooled to a room temperature in a desiccator prior to preparing the buffer. Solutions of MgCl₂ and CaCl₂ were calibrated by titration against AgNO₃ in the presence of dichromate indicator as described by Vogel (1961) cited in May-Linn and Andrew (2010) study.

12 g of Tris base was dissolved in 80 mL of nuclease-free water in the container and the mixture in the container was placed on the Hotplate Stirrer and enable at slow speed setting to ensure that there is no splashing during the process. Once enabled, a “turtle” icon was appearing on the screen and the stirring function started until the Tris-base was completely dissolved. The pH meter was adjusted slowly to 7.4 value and 6 mL concentrated HCl was added to the solution above and observed the increases in the temperature of the mixture that might affect the pH of the solution. The solution was allowed to cool to room temperature before making final adjustments to the pH using more concentrated HCl.

➤ Tris-Dissolution

12g of Tris was poured into distilled water and continue stirring until it completely dissolved. The time to dissolve Tris was 10 minutes depends on its purity and solubility. After the Tris was completely dissolved, the pH meter was adjusted by addition of HCl solution drop by drop to measure the pH value of the solution simultaneously to avoid excessive adjustment. The adjustment inspection was carried out to recheck the pH value of the solution by ensuring sufficient mixing and stirring of the solution during the adjustment process.

➤ Screening and Storage

Once the required pH value was reached, the solution was filtered into a clean container to remove insoluble substances and impurities. For long-term storage, it was stored in Tris-HCl hydrochloride buffer under refrigerated conditions to ensure stability and long-term use. The correct synthesis of Tris HCl buffer solution obtained was provided stable buffering performance, accuracy and repeatability of experimental results. During the synthesis process, laboratory's safety regulations are carefully followed to avoid accident in the laboratory.

2.2 METHODOLOGY

The methods used in this study include: Calibration of Buffer Solution by Titration; PH Determination; Thermal Stability Test; Determination of Amylases Activity; and Determination of Protease Activity

3.0 RESULT AND DISCUSSION

3.1 RESULTS

3.1.1 Tris-Buffer Basic Information

Table 3.1: Basic Information of Tris-Buffer

Test	Observations		
Parameters	Solubility	Molecular Formula	Molecular Weight
Tris-base	Water solubility	C ₄ H ₁₁ NO ₃	121.14
Acidity coefficient(pKa)	8.1(at 25°C)	Color-changing, pH range of the acid-base indicator	pH
Temperature	550 g/L (25 °C)	7 – 9	10.5-12.0 (4 m in water, 25 °C)

From Table 3.1, the tris-buffer solution locally produced was soluble in water with molecular mass of $C_4H_{11}NO_3$ and its colour changes range of the acid-base indicators of 8.1 to 9.0 pH with changing in temperature. Its minimum pH was 7 while the maximum pH was 12 at different conditions of temperature, humidity and other environmental conditions and has 121.14g molecular weight.

3.1.2 Buffer Solution by Titration

From the Table 4.2 above, the average titration value obtained was 26.40 mol/dm^3 while the average value of buffer solution used was 26.30 mol/dm^3 . The differences between the volume of buffer solution and acid used for titration was $\pm 0.10 \text{ mol/dm}^3$ less than $\pm 0.50 \text{ mol/dm}^3$ standard buffer solution. Also, the specific gravity of locally produced tris-buffer solution was 0.3180 g/mL which was slightly less than the standard solution of 0.3253 g/mL obtained from DC-Fine Chemical website. The different between the two values was 0.0073 g/ml which was also less than 0.5 g/mL level of error. Therefore, this locally tris-buffer solution produced is acceptable and can be used for scientific experiments such as biological, chemical, biochemical, and pharmaceutical experiments in the laboratory.

Table 3.2: Titration Result and Specific Gravity

Test	Observations			
Parameters	1 st Titration (Control)	2 nd Titration	3 rd Titration	Average Titration Value
Volume of Tris-buffer Solution (mol/dm^3)	26.40	26.20	26.30	26.30
Volume of Acid Used (mol/dm^3)	26.50	26.40	26.40	26.40
Average Titration Value (mol/dm^3)	± 0.10	± 0.20	± 0.10	± 0.10
Specific gravity (g/mL)	Standard oil values	Extracted oil values	MDDF	
	0.3253	0.3180	0.0073	

Note: MDDF = Mean Diameter Different

Source: The standard specific gravity of standard buffer solutions was obtained from www.dcfinechemicalsolution.com.

In Table 3.3 below, the compositions parameters of tris-buffer produced and its amount at $0.04 \text{ mol.kg-H}_2\text{O}^{-1}$ equimolar ratio against the standard were evaluated and discovered that buffer contained $0.0800 \text{ mol.kg-H}_2\text{O}^{-1}$ and $0.0765 \text{ mol.kg-solution}^{-1}$ at $0.04 \text{ mol.kg-H}_2\text{O}^{-1}$ equimolar Tris ratio while standard buffer was $0.0801 \text{ mol.kg-solution}^{-1}$. The component HCl has $0.0400 \text{ mol.kg-H}_2\text{O}^{-1}$ and $0.0383 \text{ mol.kg-solution}^{-1}$ against standard HCl of $0.0369 \text{ mol.kg-solution}^{-1}$ while NaCl component has $0.3876 \text{ mol.kg-H}_2\text{O}^{-1}$ and $0.3709 \text{ mol.kg-solution}^{-1}$ against standard HCl of $0.03810 \text{ mol.kg-solution}^{-1}$ and Na_2SO_3 component has $0.0293 \text{ mol.kg-H}_2\text{O}^{-1}$ and $0.0280 \text{ mol.kg-solution}^{-1}$ against standard Na_2SO_3 of $0.0281 \text{ mol.kg-solution}^{-1}$.

3.1.3 Composition of Tris-Buffer Solution Produced

Table 3.3: Composition of Tris-Buffer Solution

Components	Observations			
Parameters	mol. $\text{kg-H}_2\text{O}^{-1}$	mol. kg-solution^{-1}	Impurity components (%)	Standard mol. kg-solution^{-1}
Buffer	0.0800	0.0765	$\pm 0.02\%$	0.0801

HCl	0.0400	0.0382	± 0.02%	0.0369
NaCl	0.3876	0.3709	± 0.01%	0.3810
Na ₂ SO ₃	0.0293	0.0280	± 0.01%	0.0281
KCl	0.0106	0.0101	± 0.01%	0.0100
MgCl ₂	0.0547	0.0524	± 0.01%	0.0530
CaCl ₂	0.0108	0.0103	± 0.01%	0.0105

Note: (Source: Devails and Dickson, 1998: Standard (mol.kg-solution⁻¹))

The KCl component contained 0.0106 mol.kg-H₂O⁻¹ and 0.0101 mol.kg-solution⁻¹ against standard KCl of 0.0100 mol.kg-solution⁻¹, MgCl₂ component has 0.0547 mol.kg-H₂O⁻¹ and 0.0524 mol.kg-solution⁻¹ against standard MgCl₂ of 0.0530 mol.kg-solution⁻¹ while CaCl₂ component has 0.0108 mol.kg-H₂O⁻¹ and 0.0524 mol.kg-solution⁻¹ against standard CaCl₂ of 0.0105 mol.kg-solution⁻¹ while the impurities were very low. This result shows that the Tris-buffer locally produced contained appropriate amount of chemical compositions elements that make it suitable for scientific experiments analysis.

3.1.4 Reagents Preparation of 1-Litres of Tris-Buffer Solution Locally Produced at Variable PH

In Table 3.4 below, the reagents preparation for tris-buffer locally produced were measured and discovered that buffer (mol.kg-solution⁻¹) has weight of 9.517g and low impurities of ± 0.02% against standard buffer of 8.921g. There was little different between the locally buffer characterized and standard one of 0.605g. The HCl (mol.kg-solution⁻¹) weighed 39.270g, impurities of ± 0.01% and contributed 0.02 to the buffer pH against standard of 36.991g, NaCl (mol.kg-solution⁻¹) weighed 23.954 g, impurities of ± 0.01% and contribute 0.0005 to buffer pH compared to standard weight of 24.210g, Na₂SO₃ (mol.kg-solution⁻¹) weighed 4.085g, impurities of 0.100% and has 0.001 contributed to buffer pH against standard of 4.190g, KCl (mol.kg-solution⁻¹) weighed 0.775g, impurities of ± 0.01% and contributed -0.001 to the buffer pH against standard of 0.790g, MgCl₂ (1 mol.L⁻³) weighed 58.862g, impurities of ± 0.015% and contributed 0.0004 to the buffer pH against standard of 60.001g, CaCl₂ (1 mol.L⁻³) weighed 11.726g, impurities of ± 0.015% and contributed 0.0001 to the buffer pH against standard of 12.100g and deionized water (H₂O⁻) in which its weight fill to line, impurities of ± 0.0001% and contributed 0.001 to the buffer pH against standard which is always fill to line as well.

3.1.5 Thermal Stability and Self Efficacy

In subjected the tris-buffer locally produced samples produced into different temperature of room temperature, 40°C and 4°C respectively. There were no changes in colour, texture and odour of the samples in 3 different temperatures (i.e. room temperature, 40°C and 4°C). Only sample treated or stored at 40°C temperature has slight phase separation while samples at room temperature and 4°C have no phase separation changes.

Table 3.4: Reagents Formulated for Tris-Buffer Solution

Components	Observations			
	Weighed of Component (g)	Tolerable Impurities (%)	Contribution to Buffer pH	Standard mol.(g)
Buffer (mol.kg-solution ⁻¹)	9.517	± 0.02	-	8.9210
HCl (mol.kg-solution ⁻¹)	39.270	± 0.01	0.02	36.991
NaCl (mol.kg-solution ⁻¹)	23.954	± 0.01	0.0005	24.210
Na ₂ SO ₃ (mol.kg-solution ⁻¹)	4.085	± 0.100	0.001	4.190
KCl (mol.kg-solution ⁻¹)	0.775	± 0.010	-0.001	0.790
MgCl ₂ (1 mol. L ⁻³)	58.862	± 0.015	0.0004	60.001
CaCl ₂ (1 mol. L ⁻³)	11.726	± 0.015	0.0001	12.100
Deionized water (H ₂ O ⁻)	Fill to line	± 0.001	0.001	Fill to line

Note: (Source: Devails and Dickson, 1998: Standard (mol.kg-solution⁻¹))

Table 3.5: Thermal Stability Analysis

Conditions	Observations				
Temperature	Colour	Odour	Texture	Phase Separation	Result
Room Temperature (Control)	No changes observed	No changes observed	No changes observed	No changes observed	Stable
High Temperature (40°C)	No changes observed	No changes observed	No changes observed	Slight phase separation	Stable
Low Temperature (4°C)	No changes observed	No changes observed	No changes observed	No changes observed	Stable

Table 3.6: Amylase and Protease Activity

Test	Observations				
Parameters	Sample 1	Sample 2	Sample 3	MD	Analysis
Amylase Activity (U/g)	Sample 1	Sample 2		Sample 3	Mean
	0.47	0.46		0.48	0.47
Protease Activity(U/g)	Sample 1	Sample 2		Sample 3	Mean
	0.009	0.008		0.010	0.009

Note: MD = Mean Diameter;

3.1.6 Determination of Amylase Activity

The amylase activity of the tris-buffer locally produced was determined and discovered that the tris-buffer locally produced samples contained 0.47U/g, 0.46 U/g and 0.48U/g. The average mean value was 0.47U/g, this means the tris-buffer locally produced has a good amylase activity.

3.1.7 Determination of Protease Activity

The protease activity of the tris-buffer locally produced was determined. The tris-buffer locally produced samples contained 0.009U/g, 0.008 U/g and 0.010U/g. The average mean value was 0.009U/g, this means the tris-buffer locally produced has an excellent protease activity.

3.2 DISCUSSION

The finding of the study in Table 4.1 revealed that Tris-buffer solution locally produced with molecular mass of $C_4H_{11}NO_3$, its colour changes range of the acid-base indicators with changing in temperature while its minimum pH was 7 while the maximum pH was 12 at different conditions of temperature, humidity and other environmental factors. This showed that tris-buffer solution have chemical properties similar to the standard tris buffer solution. Similarly, Marco (2019) study's finding stated that tris-buffer solution has a good buffering capacity between pH 7.2 - 9.0, but it shows a relatively strong temperature dependence of the acid constant ($pK_a = 0.031 K^{-1}$). The pH increases when the solution is cooled or decreases when the solution is heated. May-Linn and Andrew (2020) revealed that Tris buffers shall pH value of not less than 7.0 and must soluble in water. Heiner (2018) also stated that chemically, primary Tris-buffer solution with three alcoholic hydroxyl groups, has a pK_a of 8.06, with effective buffering capacity in a pH range between 7.0 and 9.0.

The finding of the study in Table 4.2 showed that Tris-buffer solution locally produced in this study has average titration value of 26.40 mol/dm^3 and the specific gravity of locally produced tris-buffer solution was 0.3180 g/mL which was slightly less than the standard solution of 0.3253 g/mL and this solution produced was very active like commercial tris-buffer solution which make it acceptable for scientific experiments such as biological, chemical, bio-chemistry, chemistry and pharmaceutical experiments. According to Crag (2018), locally produced Tris-Buffer solution is used in various pharmaceutical dosage forms such as injection and infusion

solutions, eye drops, creams and gels as an excipient for stabilization. Similarly, Heiner (2018) stated that local tris-buffer solution is used in bacteriology, often in conjunction with inorganic buffers, to allow acid sensitive organisms to grow in multi strain cultures where acid producing organisms are present, while Ishi (2020) showed that locally produced tris-buffer solution is used as a drug for the treatment of metabolic acidosis, and also for the alkalization of urine in cases of poisoning with weakly acidic substances such as barbiturates.

The finding of the study in Table 4.3 showed discovered that buffer contained $0.0800 \text{ mol.kg-H}_2\text{O}^{-1}$ and $0.0765 \text{ mol.kg-solution}^{-1}$ at $0.04 \text{ mol.kg-H}_2\text{O}^{-1}$ equimolar Tris ratio while standard buffer was $0.0801 \text{ mol.kg-solution}^{-1}$ and all components amount obtained were fall in the range of standard tris-buffer solutions (i.e. the values of HCl, NaCl, Na_2SO_3 , KCl, MgCl_2 , and CaCl_2 were almost the same as the commercial tris-buffer solution). Therefore, this locally produced tris-buffer solution is suitable for scientific experiments because it consist required chemical components. This result shows that the Tris-buffer locally produced contained appropriate amount of chemical compositions elements that make it suitable for scientific experiments analysis.

The amylase and protease activity of the Tris-buffer locally produced was determined and discovered that the Tris-buffer locally produced samples contained 0.47U/g, 0.46 U/g and 0.48U/g. The average mean value was 0.47U/g, this means the Tris-buffer locally produced has a good amylase activity, while the protease activity of the sample was determined. The Tris-buffer locally produced samples contained 0.009U/g, 0.008 U/g and 0.010U/g. The average mean value was 0.009U/g, this means the Tris-buffer locally produced has an excellent protease activity.

Also, the reagents preparation for tris-buffer locally produced were measured and discovered that tris-buffer solutions have appropriate and acceptable reagents of HCl, NaCl, Na_2SO_3 , KCl, MgCl_2 , CaCl_2 and deionized water, low impurities and contributed to the buffer solutions' pH. Similarly, Irish (2020) stated that tris-base is used for biochemical, microbiological, pharmaceutical and molecular biology purposes as a buffer substance due to acceptable amount of its chemical compositions. Michael (2020) contributed to the study and opined that locally produced tris-buffer solution is an alkalizing and pH-regulating effect, i.e. it acts as a pH buffer, regulating and counteracting small changes in the pH of pharmacological solutions.

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

The findings of this study revealed that Tris-buffer solution locally produced with local materials has colour changes range of the acid-base indicators with changing in temperature while its minimum pH was 7 while the maximum pH was 12 and contained appropriate amount of chemical compositions with a good of average titration values. Therefore, the study concluded that Tris-buffer solution prepared from local materials is suitable for scientific experiments. It was also concluded that the colorimetric acid-base titration used to calibrate the HCl to the Tris-buffer solution allowed for significant saving on the local materials used for tris-buffer prepared. Generally, the study concluded that the characterization and standardization of Tris-buffer solution produced from local raw materials was successful prepared and was acceptable for biological, chemically, bio-chemically, pharmaceutically and others scientific experiments.

4.2 Recommendations

Base on the conclusion above, the study hereby recommends that:

- The study also recommends this product to laboratories especially institutions' laboratories for the scientific experiments instead of using commercial tris-buffer solution which is costly than tris-buffer solution produced from local materials.
- However, this tris-buffer solution produced from local materials should be considered by Biologists, Chemists, Bio-chemists, Pharmaceutics, and other scientists' professionals to facilitate the accuracy and efficiency of the experiments.
- Also, more research should be carried out to generate a cheaper means of tris-buffer solution.

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