Open Access Library Journal
2026, Volume 13, e15744
ISSN Online: 2333-9721
ISSN Print: 2333-9705

Experimental Assessment of the Pre-Analytical Causes of Haemolysis and Their Effects on Serum Electrolytes (Na^+, K^+, Cl^-) and Glucose among Students of Florence Nightingale Higher Institute of Health and Biomedical Sciences Bamenda

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How to cite this paper: Shalanyuy, L.H., Ndzi, S.F., Achungu, C.R., Emlah, N.V., Toboh, R.T. and Foncham, E. (2026) Experimental Assessment of the Pre-Analytical Causes of Haemolysis and Their Effects on Serum Electrolytes (Na^+, K^+, Cl^-) and Glucose among Students of Florence Nightingale Higher Institute of Health and Biomedical Sciences Bamenda. *Open Access Library Journal*, **13**: e15744.
https://doi.org/10.4236/oalib.1115776

Received: July 7, 2026
Accepted: August 24, 2026
Published: August 27, 2026

Abstract

Haemolysis remains the most frequently encountered pre-analytical error in clinical laboratories, yet its specific impact on routine biochemical analytes under local laboratory conditions in resource-limited settings like Cameroon is poorly documented. This experimental study assessed the pre-analytical causes of serum haemolysis and their effects on serum electrolytes (Na^+, K^+, Cl^-) and glucose among students of Florence Nightingale Higher Institute of Health and Biomedical Sciences, Bamenda. A total of 21 participants were recruited using convenience sampling. Venous blood samples were collected and divided into control and test portions. Haemolysis was experimentally induced through three pre-analytical manipulations: forcing blood through a needle into the collection tube (T1), vigorous shaking of blood collection tubes (T2), and excessive centrifugation speed (T3). Serum sodium, potassium, and chloride were measured by spectrophotometry, and glucose was determined using a glucometer. Data was analysed using one-way ANOVA, with statistical significance set at $p < 0.05$. Vigorous shaking produced the highest degree of haemolysis (4+), followed by forcing blood through a needle (3+), while excessive centrifugation caused only mild haemolysis (1+). Potassium concentrations dif-

fered significantly across groups ($F = 13.90$, $p < 0.001$), with the highest mean in the vigorous shaking group (5.84 ± 1.05 mmol/L) compared to the control (4.26 ± 0.87 mmol/L). Sodium ($F = 0.11$, $p = 0.953$) and chloride ($F = 0.28$, $p = 0.84$) showed no statistically significant differences across conditions. Glucose levels were likewise unaffected ($F = 0.39$, $p = 0.76$). These findings confirm that improper specimen handling, particularly vigorous shaking is a leading driver of *in vitro* haemolysis and that potassium is the analyte most vulnerable to haemolysis-induced bias, with elevations large enough to produce clinically misleading results. Sodium, chloride, and glucose remained relatively stable under the conditions studied. The study underscores the need for reinforced phlebotomy training, strict adherence to specimen handling protocols, and routine haemolysis monitoring in teaching and clinical laboratories in Cameroon.

Subject Areas

Clinical Medicine

Keywords

Haemolysis, Pre-Analytical Errors, Serum Electrolytes, Glucose, Potassium, Phlebotomy, Cameroon

1. Introduction

Over the past decade, clinical laboratory medicine has undergone a marked transformation driven by automation and advances in analytical technology, leading to a substantial reduction in analytical-phase errors and a corresponding improvement in the reliability of laboratory data [1]. However, this progress has shifted attention toward the pre-analytical phase, now recognized as the most vulnerable step in the total testing process. Pre-analytical variables, including specimen collection, handling, transport, and processing, remain insufficiently standardized in many settings and continue to exert a profound influence on the validity of laboratory results, thereby directly affecting clinical decision-making [1].

Among routine biochemical investigations, serum electrolytes (sodium, potassium, and chloride) and glucose occupy a central role in both acute and chronic patient management. These analytes guide critical interventions such as the correction of dyskalemias and the management of dysglycaemia, making their accuracy non-negotiable for patient safety [1]. Yet, the pre-analytical phase contributes disproportionately to laboratory errors, accounting for approximately 40% - 70% of all total testing process inaccuracies, with hemolysis identified as the most frequent and clinically significant source of interference [2]. Hemolysis, defined as the rupture of erythrocytes with subsequent release of intracellular contents into plasma or serum, commonly arises from preventable procedural and mechanical factors during blood collection and processing [3].

The clinical relevance of hemolysis is particularly evident in potassium meas-

urement, where even minimal erythrocyte lysis can cause substantial false elevations due to the markedly higher intracellular potassium concentration relative to plasma. This phenomenon frequently results in pseudohyperkalaemia, which may lead to unnecessary and potentially harmful interventions [4]. While sodium and chloride are generally less sensitive to hemolytic interference, their measured concentrations may still be affected depending on analytical methodology, particularly the use of indirect ion-selective electrodes and variations in plasma composition [5]. Glucose measurement also demonstrates variable susceptibility, with hemolysis-induced release of intracellular enzymes and assay-dependent factors producing device-specific analytical bias in some systems [6].

To mitigate these challenges, laboratories increasingly rely on automated hemolysis indices (H-index) as objective tools for detecting and quantifying hemolysis. These indices, endorsed by international bodies such as IFCC and CLSI, correlate with free hemoglobin concentration and support standardized decision-making regarding sample rejection or result validation [7]. Despite these advances, high hemolysis rates persist in routine practice, particularly in high-throughput clinical environments, leading to repeated sampling, diagnostic delays, increased healthcare costs, and potential patient harm [8]. Importantly, variability in phlebotomy practices, training levels, and infrastructure across settings, especially in low-resource environments, continues to exacerbate the burden of pre-analytical errors [9].

In Cameroon, despite the implementation of national laboratory quality policies and alignment with WHO-AFRO SLIPTA/SLMTA frameworks, hemolysis remains a persistent challenge due to inconsistent adherence to standard operating procedures, limited continuous training, and insufficient infrastructure support [6] [7]. Moreover, there is a critical lack of institution-specific experimental data quantifying the magnitude of hemolysis-induced bias on key biochemical analytes using locally available laboratory systems. This gap is particularly evident in Bamenda, where no experimental studies have systematically evaluated the effect of hemolysis on sodium, potassium, chloride, and glucose within teaching and diagnostic laboratories. Therefore, this study, conducted at the Florence Nightingale Higher Institute of Health and Biomedical Sciences, aims to generate locally relevant evidence on hemolysis-related analytical bias, with the goal of strengthening laboratory quality systems, refining sample rejection criteria, and improving both training and patient safety outcomes [10].

2. Methodology

This experimental laboratory-based study was conducted in Bamenda III Subdivision, Mezam Division, North West Region of Cameroon, in the Florence Nightingale Higher Institute of Health and Biomedical Sciences (FLENHIHBS) laboratory, where all sample collection, processing, and biochemical analyses were performed from January 15 to July 13, 2026. The study involved 21 venous blood samples collected from consenting FLENHIHBS students selected through con-

venience sampling, with inclusion criteria restricting participation to healthy individuals without hemolytic disorders, G6PD deficiency, or active malaria infection, and exclusion applied to those who declined consent. Eligibility was verified through a brief health assessment and participant self-reporting, complemented by review of available medical history, with malaria status confirmed by absence of current clinical symptoms and no evidence of active infection at the time of sample collection. Participants with a known history of hemolytic disorders or G6PD deficiency were excluded based on their reported medical history. Each blood sample was divided into four aliquots comprising one control sample and three test samples (T1, T2, and T3). The control aliquots were processed under optimal pre-analytical conditions, while the test aliquots were subjected to controlled hemolysis-inducing factors such as forcing blood into tubes through the needle (T1), vigorous shaking (T2), and excessive centrifugation (T3). The degree of haemolysis was assessed visually after centrifugation by comparing the colour intensity of the serum with a standardized visual haemolysis scale. Samples were graded according to the extent of visible pink to red discoloration of the serum, where 1+ represented mild haemolysis (faint pink discoloration), 3+ represented moderate haemolysis (distinct red discoloration), and 4+ represented severe haemolysis (deep red serum indicating marked red blood cell lysis). Visual grading was performed consistently by the same investigator under uniform lighting conditions to minimize observer variability. Serum was obtained by clotting and centrifugation at 2500 rpm for 5 minutes, while glucose was analyzed from fluoride oxalate samples. For glucose analysis, blood was collected separately into fluoride oxalate tubes to obtain fluoride oxalate plasma, which was used directly for glucose determination using an enzymatic electrochemical glucometer method (OneTouch Ultra 2). Serum sodium, potassium, and chloride were measured using standard spectrophotometric methods (magnesium uranyl acetate for sodium, sodium tetraphenylboron for potassium, and mercuric thiocyanate for chloride), with strict quality control measures including correct specimen handling, appropriate tube selection, timely processing, prevention of pre-analytical errors in controls, and adherence to storage protocols. Data was collected using structured questionnaires and laboratory result sheets, then analyzed using SPSS version 21, with descriptive statistics summarizing variables. Since each participant contributed measurements under all four experimental conditions (control, T1, T2, and T3), inferential analyses accounted for the repeated-measures study design. Normality of the data was assessed prior to hypothesis testing. Differences between control and individual test conditions were evaluated using paired-samples t-tests, while overall differences across the four related conditions were assessed using repeated-measures analysis of variance (repeated-measures ANOVA), followed by appropriate post hoc pairwise comparisons where significant differences were observed. Statistical significance was set at $p < 0.05$. Ethical approval was obtained from the North West Regional Delegation of Public Health, and written informed consent was secured from all participants, ensuring voluntary participation, con-

fidentiality, and anonymity throughout the study.

3. Results

3.1. Socio-Demographic Characteristics of Study Participants

Table 1 presents the socio-demographic characteristics of the study participants. Out of the 21 participants, the majority were females, accounting for 57.1% (n = 12), while males constituted 42.9% (n = 9). Regarding age distribution, most participants were within the 23 - 25 years age group, representing 61.9% (n = 13), whereas 38.1% (n = 8) were aged 18 - 23 years. Concerning the level of study, the majority of respondents were in Level 400, accounting for 61.9% (n = 13), while 38.1% (n = 8) were in Level 300. No participants were from Level 100 or Level 200 (0.0%; n = 0). With respect to the program of study, Nursing students constituted the largest proportion of participants, representing 57.1% (n = 12). This was followed by Medical Laboratory Science students at 33.3% (n = 7). Midwifery and Pharmacy Technology students each accounted for 4.8% (n = 1) of the study population.

Table 1. Socio-demographic characteristics of study participants.

Variable	Frequency (n)	Percentage (%)
Sex		
Male	09	42.9
Female	12	57.1
Age Range (Years)		
18 - 23	08	38.1
23 - 25	13	61.9
Level of Study		
Level 100	00	00.0
Level 200	00	00.0
Level 300	08	38.1
Level 400	13	61.9
Program of Study		
Medical Laboratory Science	07	33.3
Nursing	12	57.1
Midwifery	01	04.8
Pharmacy Technology	01	04.8

3.2. Major Pre-Analytical Factors Associated with Serum Hemolysis

The findings indicate that vigorous shaking produced the highest degree of hemolysis (4+), resulting in gross red blood cell destruction and dark red plasma/se-

rum. Forcing blood through a needle caused moderate hemolysis (3+), suggesting substantial mechanical damage to erythrocytes during sample handling. In contrast, excessive centrifugation speed resulted in only slight hemolysis (1+), indicating minimal red blood cell damage compared to the other methods as indicated in Figure 1 below.

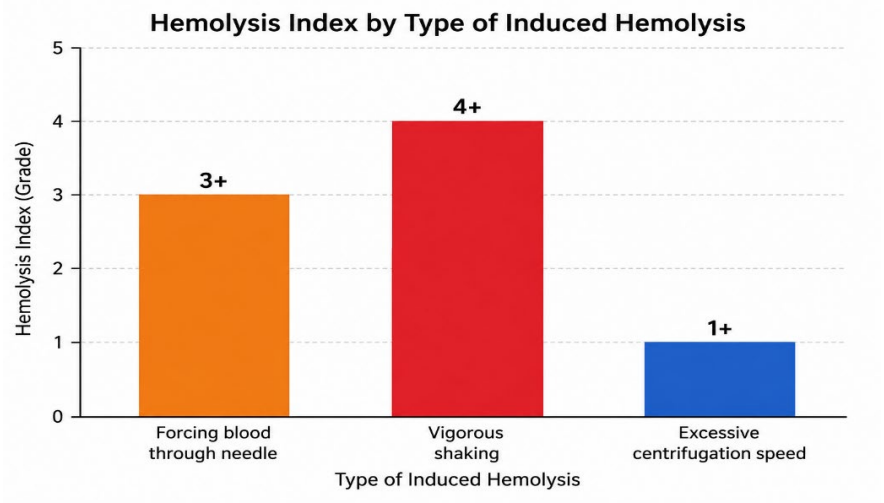
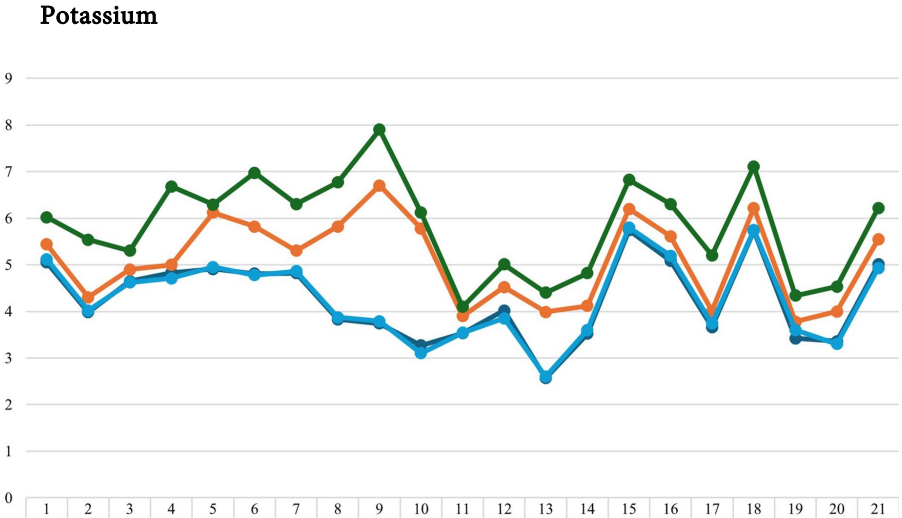


Figure 1. Major pre-analytical factors associated with serum hemolysis.

3.3. Effect of Hemolysis on Serum Electrolyte Concentrations (K^+, Na^+, Cl^-)



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
POTASIUM(mmol/L) Control	5.05	3.98	4.65	4.83	4.91	4.81	4.82	3.83	3.74	3.27	3.53	4.02	2.57	3.52	5.74	5.09	3.66	5.73	3.42	3.36	5.01
POTASIUM(mmol/L) T1	5.44	4.3	4.90	5.00	6.12	5.82	5.3	5.82	6.7	5.78	3.90	4.52	3.99	4.12	6.20	5.61	4.01	6.22	3.78	4.0	5.55
POTASIUM(mmol/L) T2	6.02	5.54	5.30	6.68	6.29	6.97	6.30	6.77	7.9	6.12	4.1	5.01	4.40	4.82	6.82	6.30	5.2	7.11	4.34	4.53	6.22
POTASIUM(mmol/L) T3	5.12	4.02	4.62	4.71	4.95	4.78	4.86	3.87	3.79	3.10	3.54	3.85	2.60	3.60	5.80	5.19	3.74	5.75	3.60	3.30	4.93

Figure 2. Effect of hemolysis on serum potassium.

The potassium concentration increased in all induced hemolysis conditions compared with the control samples, although the magnitude of increase varied according to the type of hemolysis. Samples subjected to vigorous shaking (T2)

showed the highest potassium values, ranging from 4.10 to 7.90 mmol/L, indicating the greatest degree of potassium release from erythrocytes due to severe mechanical disruption of cell membranes. Forcing blood through a needle (T1) also resulted in a marked elevation of potassium concentrations (3.78 - 6.70 mmol/L) compared with the control group (2.57 - 5.74 mmol/L), demonstrating moderate hemolysis-induced potassium leakage. In contrast, excessive centrifugation speed (T3) produced potassium values (2.60 - 5.80 mmol/L) that were only slightly different from the control samples, suggesting minimal hemolytic effect (See **Figure 2**).

Potassium concentrations differed significantly among the study groups ($F = 13.90$, $p < 0.001$). The highest potassium level was observed in samples subjected to vigorous shaking (T2) (5.84 ± 1.05 mmol/L), followed by samples forced through a needle (T1) (5.10 ± 0.92 mmol/L). Potassium concentrations in the excessive centrifugation group (T3) (4.27 ± 0.88 mmol/L) were comparable to the control group (4.26 ± 0.87 mmol/L) (See **Table 2**).

Table 2. Effect of different types of induced hemolysis on serum potassium concentration.

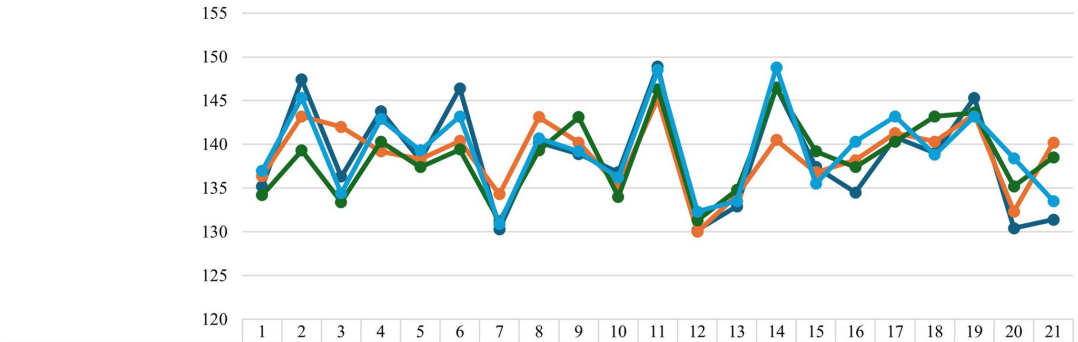
Test	Mean ± SD Potassium (mmol/L)
Control	4.26 ± 0.87^{c}
T1	5.10 ± 0.92^{b}
T2	5.84 ± 1.05^{a}
T3	4.27 ± 0.88^{c}
F-value	**13.90**
p-value	**<0.001**

Values are expressed as mean ± standard deviation (SD). Means with different superscript letters ($^{a, b, c, d}$) differ significantly from each other, whereas means sharing the same superscript letter do not differ significantly, according to the post hoc multiple-comparison test following one-way analysis of variance (ANOVA) ($p < 0.05$).

Sodium

The sodium concentrations showed relatively small variations across the different hemolysis-inducing conditions compared with the control samples, indicating that sodium measurement was minimally affected by hemolysis. The control samples had sodium values ranging from 130.2 to 148.9 mmol/L. Samples subjected to forcing blood through a needle (T1) showed values ranging from 130.0 to 145.2 mmol/L, while those exposed to vigorous shaking (T2) ranged from 131.2 to 146.4 mmol/L. Excessive centrifugation speed (T3) produced sodium concentrations ranging from 130.9 to 148.8 mmol/L. Although slight increases and decreases were observed among individual samples, the magnitude of change was considerably smaller than that observed for potassium. Vigorous shaking (T2) tended to produce slightly lower sodium values in some samples, whereas excessive centrifugation speed (T3) yielded values that were generally comparable to the control group (See **Figure 3**).

There was no statistically significant difference in sodium concentrations among the control group and the different hemolysis-induced groups (F = 0.11, p = 0.953). The mean sodium concentrations ranged from 138.47 ± 4.46 mmol/L in the vigorous shaking group (T2) to 139.29 ± 5.07 mmol/L in the excessive centrifugation group (T3). Since all groups shared the same superscript (a), post hoc comparisons indicated no significant differences between any of the groups (See Table 3).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Sodium(mmol/L) Control	135	147	136	144	138	146	130	140	139	137	149	130	133	147	137	135	141	139.	145	130	131
Sodium(mmol/L) T1	136	143	142	139	138	140	134	143	140	135	145	130.	134	141	137	138	141	140	143	132	140
Sodium(mmol/L) T2	134	139	133	140	137	139	131	139	143	134.	146	131	135	146	139	137	140	143	144	135	139
Sodium(mmol/L) T3	137	145	134	143	139	143	131	141	139	136	149	132	134	149	136	140	143	139	143	138	134

Figure 3. Effect of hemolysis on serum Sodium.

Table 3. Effect of different types of induced hemolysis on serum sodium concentration.

Test	Mean ± SD Sodium (mmol/L)
Control	138.61 ± 5.98[a]
T1	138.80 ± 3.95[a]
T2	138.47 ± 4.46[a]
T3	139.29 ± 5.07[a]
F-value	**0.11**
p-value	**0.953**

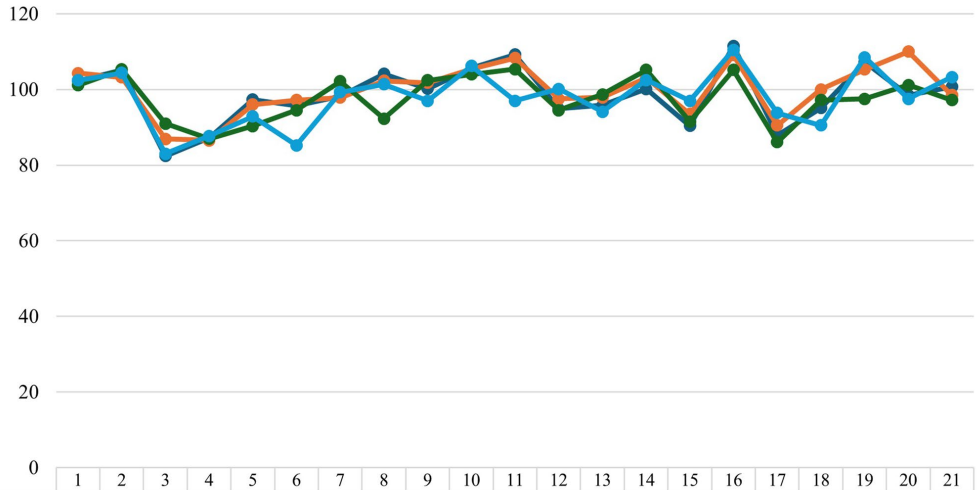
Values are expressed as mean ± standard deviation (SD). Means with different superscript letters ([a, b, c, d]) differ significantly from each other, whereas means sharing the same superscript letter do not differ significantly, according to the post hoc multiple-comparison test following one-way analysis of variance (ANOVA) ($p < 0.05$).

Chloride

The chloride concentrations showed only modest variations across the different hemolysis-inducing conditions when compared with the control samples, suggesting that chloride measurement was relatively stable despite hemolysis. The control samples had chloride values ranging from 82.4 to 111.5 mmol/L. Samples subjected to forcing blood through a needle (T1) showed chloride concentrations ranging from 86.5 to 110.0 mmol/L, while vigorous shaking (T2) produced values ranging from 86.1 to 105.4 mmol/L. Excessive centrifugation speed (T3) yielded

chloride concentrations ranging from 83.0 to 110.5 mmol/L. Although some fluctuations were observed among individual samples, the overall chloride values remained within ranges comparable to those of the control group. Vigorous shaking (T2) tended to produce slightly lower chloride concentrations in several samples, whereas forcing blood through a needle (T1) and excessive centrifugation speed (T3) resulted in values that were generally similar to those of the control samples (Figure 4).

There was no statistically significant difference in serum chloride concentrations among the control and hemolysis-induced groups ($F = 0.28$, $p = 0.84$). Mean chloride levels ranged from 97.82 ± 7.02 mmol/L in the excessive centrifugation group (T3) to 99.77 ± 6.98 mmol/L in the T1 group. All groups shared the same superscript (a), indicating no significant differences between them (Table 4).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Chloride Control	102.3	105.1	82.4	87.2	97.3	95.7	98.22	104.1	100.2	105.6	109.2	94.9	95.8	100.1	90.4	111.5	87.9	95.09	107.2	98.4	100.7
Chloride T1	104.3	103.2	86.93	86.5	96.02	97.2	97.87	102.3	101.8	105.4	108.3	97.5	97.9	103.0	93.5	109.1	90.5	100.0	105.3	110	98.5
Chloride T2	101.1	105.3	91.0	86.97	90.3	94.5	102.2	92.3	102.4	104	105.4	94.5	98.7	105.2	91.5	105.2	86.1	97.2	97.5	101.2	97.2
Chloride T3	102.4	104.4	83	87.71	92.9	85.2	99.3	101.4	97	106.2	97.0	100.1	94.1	102.5	97.0	110.5	93.8	90.5	108.5	97.5	103.3

Figure 4. Effect of hemolysis on serum chloride.

Table 4. Effect of different types of induced hemolysis on serum chloride concentration.

Test	Mean ± SD Chloride (mmol/L)
Control	98.54 ± 7.79[a]
T1	99.77 ± 6.98[a]
T2	98.08 ± 7.41[a]
T3	97.82 ± 7.02[a]
F-value	**0.28**
p-value	**0.84**

Values are expressed as mean ± standard deviation (SD). Means with different superscript letters ([a, b, c, d]) differ significantly from each other, whereas means sharing the same superscript letter do not differ significantly, according to the post hoc multiple-comparison test following one-way analysis of variance (ANOVA) ($p < 0.05$).

3.4. Combined Results

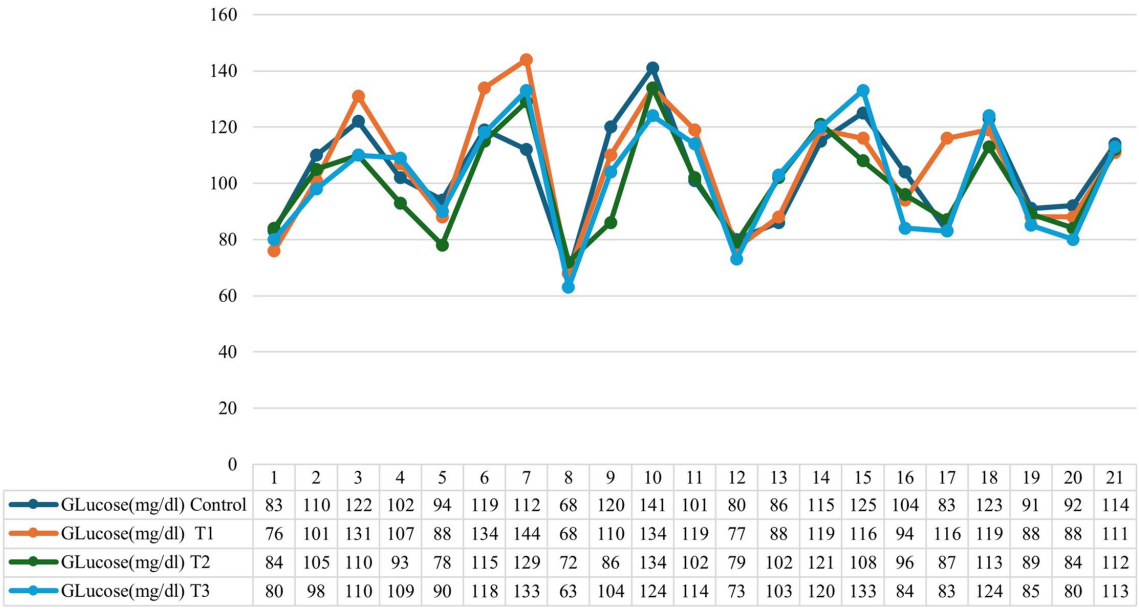
Potassium showed a statistically significant increase across groups ($p < 0.001$), with the highest concentration observed in T2 (vigorous shaking). This indicates that potassium is highly sensitive to hemolysis, consistent with release from intracellular compartments. Sodium levels remained stable across all groups ($p = 0.953$), indicating that hemolysis does not meaningfully affect serum sodium measurement under the experimental conditions. Chloride concentrations also showed no significant differences ($p = 0.840$), confirming that chloride is not significantly influenced by hemolysis induction methods (See Table 5).

Table 5. Effect of different types of induced hemolysis on serum potassium, sodium and chloride concentrations.

Parameter	Control	T1	T2	T3	F-value	p-value
Potassium (mmol/L)	4.26 ± 0.87^{c}	5.10 ± 0.92^{b}	5.84 ± 1.05^{a}	4.27 ± 0.88^{c}	13.90	<0.001
Sodium (mmol/L)	138.61 ± 5.98^{a}	138.80 ± 3.95^{a}	138.47 ± 4.46^{a}	139.29 ± 5.07^{a}	0.11	0.953
Chloride (mmol/L)	98.54 ± 7.79^{a}	99.77 ± 6.98^{a}	98.08 ± 7.41^{a}	97.82 ± 7.02^{a}	0.28	0.840

Values are expressed as mean ± standard deviation (SD). Means with different superscript letters ($^{a, b, c, d}$) differ significantly from each other, whereas means sharing the same superscript letter do not differ significantly, according to the post hoc multiple-comparison test following one-way analysis of variance (ANOVA) ($p < 0.05$).

3.5. Effect of Hemolysis on Plasma Glucose Levels



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
GLucose(mg/dl) Control	83	110	122	102	94	119	112	68	120	141	101	80	86	115	125	104	83	123	91	92	114
GLucose(mg/dl) T1	76	101	131	107	88	134	144	68	110	134	119	77	88	119	116	94	116	119	88	88	111
GLucose(mg/dl) T2	84	105	110	93	78	115	129	72	86	134	102	79	102	121	108	96	87	113	89	84	112
GLucose(mg/dl) T3	80	98	110	109	90	118	133	63	104	124	114	73	103	120	133	84	83	124	85	80	113

Figure 5. Effect of hemolysis on plasma glucose.

The glucose concentrations demonstrated noticeable variability across the different hemolysis-inducing conditions when compared with the control samples, suggesting a moderate influence of specimen handling on measured glucose levels.

The control group showed glucose values ranging from 68 to 141 mmol/L. Samples subjected to forcing blood through a needle (T1) ranged from 68 to 144 mmol/L, while vigorous shaking (T2) produced values ranging from 72 to 134 mmol/L. Excessive centrifugation speed (T3) showed glucose concentrations ranging from 63 to 133 mmol/L. T1 tended to show slightly higher glucose readings in several instances compared to the control, whereas T2 and T3 displayed mixed patterns of both increases and decreases depending on individual samples. Despite these fluctuations, the general distribution of glucose values remained broadly comparable across all conditions, indicating that hemolysis-related pre-analytical factors may introduce variability but do not consistently cause a unidirectional bias in glucose measurement (See **Figure 5**).

There was no statistically significant difference in glucose levels among the four experimental groups ($F = 0.39$, $p = 0.76$). Although minor variations in mean glucose concentrations were observed across the groups, these differences are not statistically meaningful. This indicates that the different treatments did not have a measurable effect on plasma glucose concentration under the conditions of this study (See **Table 6**).

Table 6. Effect of different treatments on plasma glucose concentration.

Test	Mean ± SD Glucose (mg/dL)
Control	104.05 ± 18.44[a]
T1	106.10 ± 21.26[a]
T2	99.95 ± 17.24[a]
T3	101.95 ± 20.40[a]
F-value	**0.39**
p-value	**0.76**

Values are expressed as mean ± standard deviation (SD). Means with different superscript letters ([a, b, c, d]) differ significantly from each other, whereas means sharing the same superscript letter do not differ significantly, according to the post hoc multiple-comparison test following one-way analysis of variance (ANOVA) ($p < 0.05$).

4. Discussion

The present study demonstrated that the degree of hemolysis varied according to the type of pre-analytical error induced, with vigorous shaking producing the most severe hemolysis (4+), followed by forcing blood through a needle (3+), while excessive centrifugation speed resulted in only mild hemolysis (1+). These findings confirm that mechanical trauma during specimen collection and handling is a major contributor to *in vitro* hemolysis. Vigorous agitation and the application of excessive pressure during blood transfer are known to disrupt erythrocyte membranes, causing leakage of intracellular constituents into serum. Previous studies have similarly identified inappropriate specimen handling, syringe-to-tube transfer, prolonged or forceful aspiration, and improper mixing as leading

causes of hemolysis in clinical laboratories, particularly where phlebotomy practices are not fully standardized [2] [3]. The findings further support reports that most laboratory errors occur during the pre-analytical phase and are largely preventable through strict adherence to standard operating procedures, proper staff training, and continuous quality improvement programs [1] [7] [9] [11].

The study showed that potassium concentration increased significantly with increasing severity of hemolysis ($F = 13.90$, $p < 0.001$), with the highest mean value observed in vigorously shaken samples (5.84 ± 1.05 mmol/L), followed by samples forced through a needle (5.10 ± 0.92 mmol/L), whereas excessively centrifuged samples were comparable to the control group. This observation is consistent with the well-established physiological distribution of potassium, where erythrocytes contain potassium concentrations many times higher than plasma. Consequently, rupture of red blood cells releases intracellular potassium into the extracellular compartment, producing pseudohyperkalaemia rather than reflecting the patient's true physiological status [4]. Similar findings have been reported in several experimental and clinical studies, which consistently demonstrate that potassium is the electrolyte most susceptible to hemolysis-induced analytical interference and that the magnitude of false elevation correlates with the severity of hemolysis [11]-[13]. These findings reinforce current laboratory recommendations that visibly hemolyzed specimens should be rejected or interpreted with caution when potassium measurement is requested to prevent inappropriate clinical management [7] [12].

Sodium concentrations remained remarkably stable across all experimental conditions, with no statistically significant difference between the control and hemolyzed samples ($F = 0.11$, $p = 0.953$). The minimal variation observed suggests that sodium measurement is relatively resistant to interference from mechanical hemolysis under the conditions employed in this study. This finding agrees with previous reports indicating that because intracellular sodium concentration is lower than extracellular concentration, erythrocyte rupture contributes very little additional sodium to serum, resulting in negligible analytical bias [5]. Furthermore, several investigations have shown that although severe hemolysis may occasionally produce minor analytical variation depending on the assay principle, particularly with indirect ion-selective electrode methods, such changes are generally not clinically significant [5] [11] [14]. The present findings therefore support the view that sodium estimation remains reliable despite mild to moderate hemolysis.

Chloride concentrations did not differ significantly among the study groups ($F = 0.28$, $p = 0.840$), indicating that chloride measurement is largely unaffected by the degree of hemolysis induced in this experiment. Although slight fluctuations in chloride values were observed, these remained within expected biological variation and were not statistically or clinically meaningful. These findings are consistent with previous studies demonstrating that chloride is relatively insensitive to hemolytic interference because the intracellular and extracellular chloride con-

centrations are comparatively similar, thereby limiting the impact of erythrocyte rupture on measured serum levels [5] [11] [15]. Collectively, the results indicate that while hemolysis produces a pronounced and clinically important effect on potassium measurement, its influence on sodium and chloride is minimal. This emphasizes the importance of recognizing hemolysis as a critical pre-analytical variable, particularly when interpreting potassium results, while sodium and chloride measurements can generally be interpreted with greater confidence in mildly hemolyzed specimens [7] [12].

The present study found no statistically significant effect of hemolysis on plasma glucose concentration ($F = 0.39$, $p = 0.76$), despite minor fluctuations observed among the different hemolysis-induced groups. These findings suggest that the pre-analytical conditions evaluated in this study did not produce sufficient analytical interference to alter glucose measurements significantly. Similar observations have been reported in previous studies, where mechanical hemolysis had little or no clinically significant effect on plasma glucose when samples were processed promptly after collection [6] [16]. However, other investigators have noted that prolonged delays in sample processing or severe hemolysis may reduce glucose concentrations due to continued glycolysis and the release of intracellular enzymes, making the extent of interference dependent on specimen handling, storage conditions, and the analytical method employed [6] [17]-[19]. Therefore, although glucose measurement appeared relatively stable under the present experimental conditions, adherence to recommended specimen collection and prompt processing remains essential to ensure accurate glucose estimation [2] [7].

5. Conclusion

This study demonstrated that the severity of hemolysis depends on the type of pre-analytical error, with vigorous shaking causing the greatest degree of hemolysis, followed by forcing blood through a needle, while excessive centrifugation produced only minimal hemolysis. Hemolysis had a significant effect on serum potassium concentration, resulting in falsely elevated values, whereas sodium, chloride, and glucose concentrations were not significantly affected under the experimental conditions.

6. Study Limitations

The study was limited by its relatively small sample size and inclusion of participants from a single institution, which may limit the generalizability of the findings. In addition, only three experimentally induced pre-analytical causes of hemolysis were investigated, while other common sources of hemolysis encountered in routine clinical practice were not evaluated.

Conflicts of Interest

The authors declare no conflicts of interest.

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