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Der Chemica Sinica, 2015, 6(3):93-99

Der Chemica Sinica
Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

# Potentiometric and spectrophotometric determination of phosphoric acid in some beverages

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**ABSTRACT**

*The Potentiometric and spectrophotometric determination of phosphoric acid content was conducted on three batches of selected beverages: Regular and Light Coke, Regular and Light Pepsi, Smirnoff Ice and 7 Up with 7 Up as control. The phosphoric acid content (mg P/L) in the three batches of these beverages using potentiometric method were: Light Coke - 136.9±5.9, 139.500±0, 139 .500±0; Regular Coke - 183.4±3.9, 175.2±0.8, 174.4± 2.3; Light Pepsi - 170.5±2.1, 172.8±3.6, 164.6±2.0; Regu lar Pepsi - 139.8±4.5, 141.6±3.1, 140.0±0.9; Smirno ff Ice - 2244.9±44.7, 2166.9±40.6, 2087.3±28.6. The results of the spectrophotometric method were respectively: Light Coke - 226.7±3.7, 207.2±1.8, 224.6±2.0; Regular Cok e - 218.4±2.7, 271.8±3.3, 242.7±1.6; Light Pepsi - 220.4±2.4, 252.9±2.3, 254.9±1.2; Regular Pepsi - 237.3±1.4, 25 6.2±1.2, 255.6±1.8; Smirnoff Ice – 916.1±25.5, 969. 1±10.7, and 923.2±19.7. 7 Up however, did not contain phosp horic acid. Analyses of variance using Student's t test showed that at 0.05 level, there was no significant statistical difference between the two methods employed as* $T_{cal}<T_{tab}$ *(0.756 < 2.13; 0.651 < 2.13 and 0.642 < 2.13 respectively). The Kruskal-Wallis H test revealed that at 0.05 level, there was no significant statistical difference in the levels of phosphoric acid in the three batches of Light Coke, Regular Pepsi, Light Pepsi and Smirnoff Ice as* $H_{cal}<H_{tab}$ *(5.96 <5.99; 5.49 <5.99; 5.96 <5.99 and 5.49 <5.99 respectively). There was however, a significant difference in phosphoric acid levels in Regular Coke as* $H_{cal}>H_{tab}$ *(7.20 > 5.99). The extremely low pH values of the beverages (2.15 – 2.85) may account for the claims of authors of being associated with cariogenicity. The phosphoric acid contents of these beverages (207.241±1.751 – 969.147±10.743 mg.P/L) pose some health concern.*

**Keywords:** phosphoric acid, beverages, potentiometric, spectrophotometric, health concern.

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## INTRODUCTION

People tend to consume large amounts of beverages most especially soft drinks like colas, diet sodas, sports drinks, beer etc. Soft drinks, for example, have been shown to be associated with a number of health risks or diseases [1-3]. Phosphoric acid which is one of the components of soft drinks has been associated with most of these health risks [4]. Additionally, there are limited data and regulation for phosphoric acid in these drinks in Nigeria. The National Agency for Food and Drug Administration and Control (NAFDAC) Soft Drinks Regulations [5] lacks an active position on the use or otherwise, of phosphoric acid and its permissible levels in soft drinks. Furthermore, some commercially available sports drinks have numerous other non – traditional ingredients; the use of phosphoric acid in beverage drinks by manufacturers is often due to its cheap nature and desirable effects regardless of attendant negative health concerns on humans.

Most food additives are considered safe, however, some have been found to be associated with adverse reactions [6]. Over the last four decades a virtual tome of information has been published linking soft drink consumption to a rise in health related issues. Evidence show an alarming rise in deficiency of calcium and other minerals resulting to

bone fractures in females. It is generally agreed that nutritional factors are important for the development of osteoporosis. Among several negative factors for bone formation are sodium, protein, caffeine, oxalate, fibre, phytate, and increased acid load, whereas calcium, vitamin D, salads, herbs, and vegetables seem to be bone promoting factors. Also alkali buffers, whether bicarbonate, vegetables, or fruits, can reverse the urinary calcium loss [7].Wyshak and Frisch [8] reported an increasing and strong association between cola beverage consumption and bone fractures in girls. Another report by Wyshak[9] indicated that cola beverages were 'highly associated with bone fracture'. The Framingham Osteoporosis study [10] indicated that cola, but not carbonated non-cola beverages, was associated with osteoporosis in women and that caffeine and phosphoric acid may adversely affect bone from the list of ingredients that make up soft drinks. In particular, phosphoric acid was shown to interfere with calcium absorption and to contribute to imbalance that leads to additional loss of calcium.

Chronic kidney disease and tooth erosion have also been shown to be associated with consumption of beverages containing phosphoric acid. A study[11] found that the risk of chronic kidney disease was doubled when participants consumed two or more colas a day. The researchers possibly attributed the findings to phosphoric acid. Researchers at the University of Illinois and Southern Illinois found that cola products containing phosphoric acid elicited an average enamel loss of 3.65% which was proportional to the length of exposure to the beverage [12].

The present study assessed the suitability of spectrophotometric and potentiometric methods for determining phosphoric acid in soft drinks. Results from this study could be useful to relevant organizations in Nigeria and environs. The data from this study could be used for quality assurance with particular reference to consistency in the levels of phosphoric acid in these beverages. It is also expected that the study would educate the general public on the dangers or otherwise, of consuming large volumes of beverages containing phosphoric acid.

## MATERIALS AND METHODS

### Sample collection and pre-treatment

Judgmental sampling technique was adopted for the study. Samples were collected from the target population (beverages) using available information about the analyte's (phosphoric acid) distribution within the population(beverages)[13], since only beverages containing phosphoric acid were collected as indicated on the label. Three brands of beverages were bought from the market in Makurdi – Nigeria ($7^{o}45'50"N$, $8^{o}32'10"E$) between October 2012 and February 2013. These consisted of two soft drinks [Coke (Regular and Light), Pepsi (Regular and Light)] and one mixed drink (Smirnoff Ice). A non-phosphoric acid containing drink (7 Up) was also collected to act as a control. Three batches of each product were collected giving a total of 18 samples.To remove the carbon (IV) oxide, approximately 100 mL of each sample was measured into a flask and heated under reflux for 20 minutes [14].

### Determination of phosphoric acid by potentiometric titration

The concentration of phosphoric acid was determined by simple acid – base titration. The decarbonated samples were each titrated with NaOH solution and the pH of the titration mixture was monitored using a pH meter. Hydrogen ions from the first dissociation of phosphoric acid react with hydroxide ions from the NaOH in a one-to-one ratio in the overall reaction:

$$H_3PO_4\,(aq) + OH^-\,(aq) \rightarrow H_2PO_4^-\,(aq) + H_2O\,(l) \qquad (1)$$

The volume of titrant (NaOH) at equivalence point was used to estimate the concentration of $H_3PO_4$ acid in each sample. The procedure for titration described by Murphy [14] was adopted. The equivalence point ($V_{eq}$) was determined from the experimental observations employing analytical (or derivative) methods consisting of plotting first and second derivative curves [i.e.ΔpH/ΔVagainst V and $\Delta^2 pH/\Delta V^2$ against V respectively] [15], where V is the volume of titrant (mL).

### Determination of phosphoric acid by spectrophotometric method

The molybdenum blue method was adopted for this determination employing ascorbic acid in the presence of potassium antimonyl tartrate as the reducing agent as described by Murphy and Rilley[16]. In an acidic medium, phosphates bond with ammonium molybdate to form ammonium phosphomolybdate. With the reducing agent (ascorbic acid), ammonium phosphomolybdate is reduced to form a bluish-purple coloured heteropolymolybdenum (V) complex also known as 'molybdenum blue'. Phosphoric acid reacts with ammonium heptamolybdate to produce ammonium phosphomolybdate according to the equation:

$$7H_3PO_4+12(NH_4)_6Mo_7O_{24}\cdot 4H_2O+25H_2SO_4 \rightarrow 7(NH_4)_3PMo_{12}O_{40}+25(NH_4)_2SO_4+83H_2O+NH_4OH \qquad (2)$$

The ammonium phosphomolybdate is then reduced by ascorbic acid in the presence of potassium antimonyl tartrate to produce the bluish-purple coloured heteropolymolybdenum (V) complex also known as 'molybdenum blue' according to the equation:

$$(PO_4 \bullet Mo_{12}^{VI}O_4 \bullet Mo_{12}^{VI}O_{36})^{3-} + 4e^- \xrightarrow{\text{(potassium antimonyl tartrate, } H_2C_6H_6O_6)} (PO_4 \bullet Mo_4^{V}Mo_8^{VI}O_{36})^{7-} \quad (3)$$

The intensity of the blue colouration is proportional to the concentration of phosphates.All samples were diluted quantitatively 50-fold except Smirnoff Ice and the blind which were diluted 100-fold prior to use. In addition, Smirnoff Ice samples were further diluted 2 – fold whereas the blind was further diluted 3-fold after colour development.

**Standard stock solution and standard solutions for calibration curve**

A solution containing 1 g P/L was prepared by dissolving 4.4158 g dry $KH_2PO_4$ in 1 L of distilled water. A working standard was prepared by diluting 10 mL of the stock solution to 100 mL, containing 100 mg P/L. Standard solutions containing 1.0, 2.0, 3.0, 4.0 and 5.0 mg P/L were prepared by diluting 1.0, 2.0, 3.0, 4.0 and 5.0 mL of the working standard to 100 mL respectively. The reagents used for colour development were sulphuric acid(5 N), ammoniummolybdate (20 g/500 mL), ascorbicacid (0.1 M), potassiumantimonyl tartrate (1mg Sb/mL). The mixed reagent was prepared by thorough mixing of 125 mL of 5 N sulphuric acid and 37.5 mL of ammonium molybdate, followed by addition of 75 mL of ascorbic acid solution and 12.5 mL of potassium antimonyl tartrate solution. This reagent was prepared as required as it does not keep for more than 24 hours.

A 40-mL aliquot of the beverage sample was pipetted into a 50-mL calibrated flask and 8 mL of the mixed reagent added. The contents were diluted to volume with distilled water, and mixed well. After I0 minutes, the absorbance of the solution was measured at 882 nm using a standard quartz cell in a UV-Visible spectrophotometer (Jenway 6305). The reagent blank was determined in the same manner using freshly distilled water and used to calibrate the spectrophotometer.

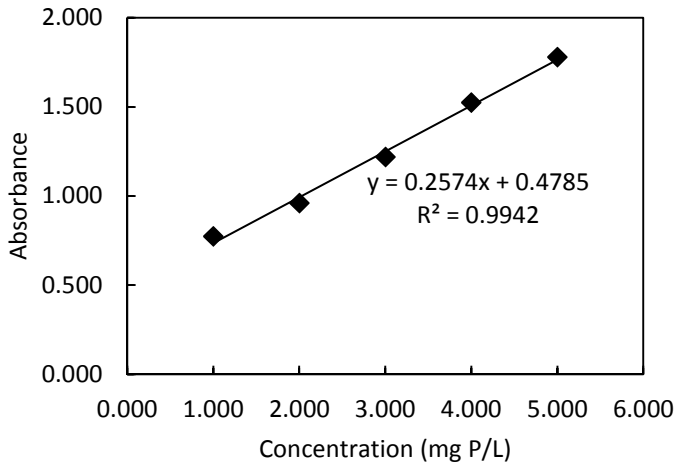


**Figure 1: Calibration curve for UV-Visible spectrophotometric determination of phosphoric acid**

**Efficiency check and data analysis**

The efficiency of the method was checked with a sample containing 1,550 mg P/L solution of phosphoric acid. This solution was used as standard/blind for the potentiometric method and blind for the spectrophotometric method. The efficiency of the pH meter (Oakton pH/con 510) and UV-Visible spectrophotometerwere then calculated using the relationship:

$$\text{Efficiency} = \frac{\text{Estimated concentration of phosphorus}}{\text{Actual concentration of phosphorus}} \times 100 \quad (4)$$

The data obtained were subjected to statistical analyses. Variations of differences between batches of the same brand of beverage were tested using the Kruskal-Wallis H test while the comparison of methods was done with the use of analysis of variance (Students' t-test) [17].

## RESULTS AND DISCUSSION

### Results of potentiometric titrations

The samples were coded using a two-digit number in which the first digit represents the batch of product while the second digit represents the number of determination of the batch and results for each batch were obtained in triplicate. The potentiometric titration curves of the beverage samples and blind (standard phosphoric acid) are as presented in Figure 2(A-F). The endpoints evaluated through first and second derivatives are as presented in Table 1. The concentration of phosphoric acid in the beverages sampled is recorded in Table 2.

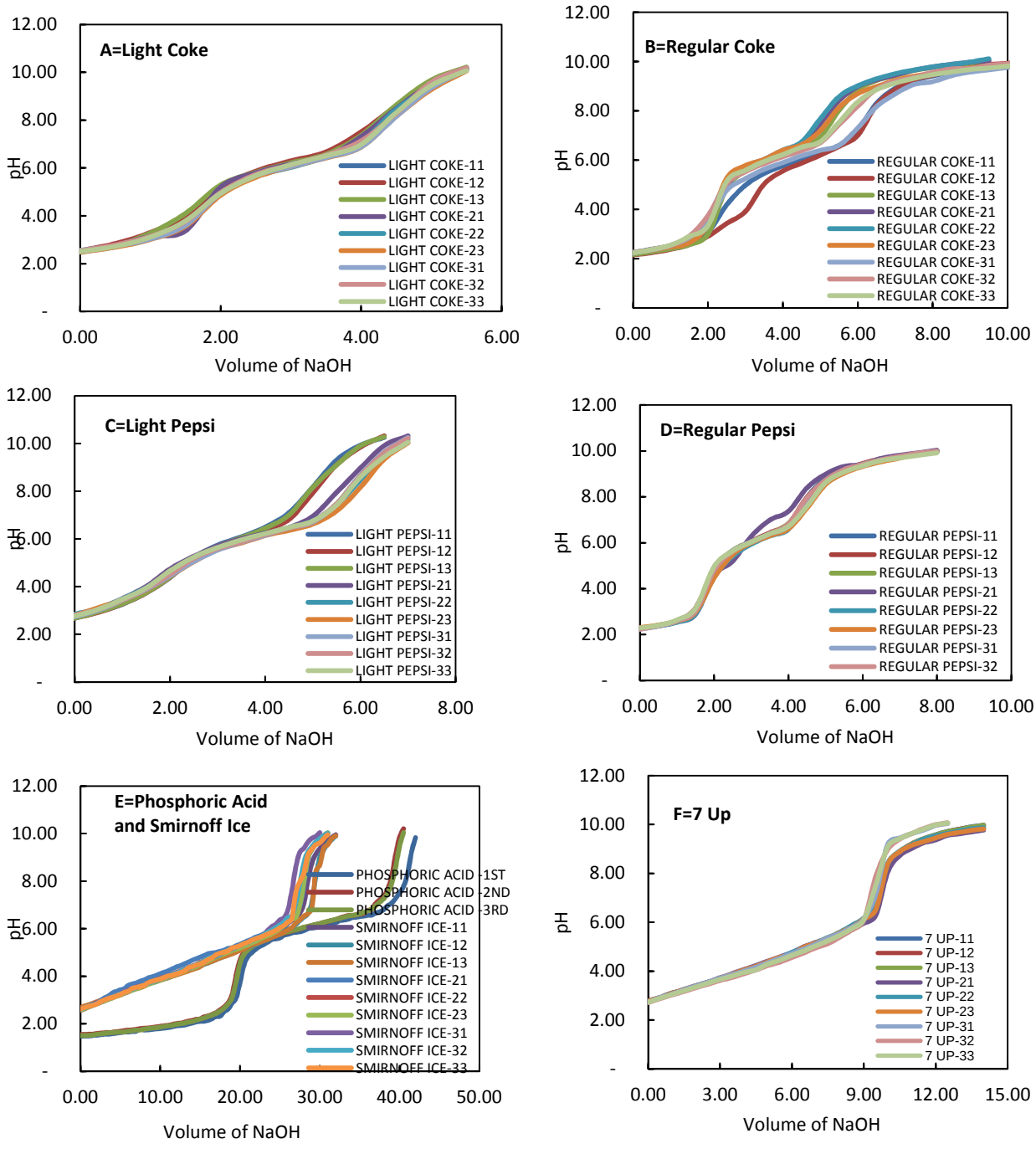


**Figure 2: Potentiometric titration curves for beverage samples and phosphoric acid**

Acids that contain more than one acidic (ionisable) proton (hydrogen) dissociate stepwisely, one proton is lost at a time. This will only be true when the successive dissociation constants ($K_a$) are different by a factor large enough, and when all of the acidic species are strong enough [18]. Phosphoric acid has $K_a$values of 7.11 $\times 10^{-3}$, $6.32 \times 10^{-8}$ and $4.5 \times 10^{-13}$that are different by a large enough factor to allow it to react with a strong base in a stepwise fashion [13].

The titration curves in Figure2(A-D) for Light Coke, Regular Coke, Light Pepsi and Regular Pepsi are characteristic of phosphoric acid as compared to the titration curve for the standard phosphoric acid shown in Figure 2(E), with p$K_{a1}$ of 2.15. Citric acid which is another acid that is commonly used as an additive in beverages with p$K_{a1}$ of 3.15 comparable to phosphoric acid, has $K_a$ values of $7.1\times10^{-4}$, $1.7\times10^{-5}$, $6.4\times10^{-6}$, that would not give such characteristic curve since the difference in the successive dissociation constants are not large enough to allow a stepwise removal of its protons as shown in Figure 2(F); 7 Up contains citric acid. The titration curve for Smirnoff Ice did not appear to be characteristic of phosphoric acid as shown in Figure 2(E). This may however be due to the removal of first protons during production as a result of pH correction leaving its conjugate base $H_2PO_4^{2-}$, the presence of citric acid or salt of phosphoric acid, as the case may be. This could further be rationalized by the decrease in the acid dissociation constant of phosphoric acid from $K_{a1}$ to $K_{a2}$ ($7.11\times10^{-3}$– $6.32\times10^{-8}$) implying that the successive proton is harder to remove. Consequently, $H_3PO_4$ is a stronger acid than $H_2PO_4^-$. For all the beverage samples, the initial pH values range from 2.15 – 2.85 whereas the standard phosphoric acid had initial pH values between 1.48 – 1.55 shown in Figure2(A-F). This is a further justification that something must have been responsible in raising the pH values upwards. It then follows that if phosphoric acid is core subject matter in any determinations in beverages, the titration curves are best qualitative in nature. Therefore the end points elucidated from the titrations for 7 Up and Smirnoff Ice may not likely be associated with the first proton from phosphoric acid, although that for 7 Up could be associated with titrable acidity [19].

**Table 1: End points of the beverages from potentiometric titrations**

| Sample | End point (mL of NaOH) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 11 | 12 | 13 | 21 | 22 | 23 | 31 | 32 | 33 |
| **Light Coke** | 1.85 | 1.75 | 1.70 | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 |
| **Regular Coke** | 2.36 | 2.42 | 2.32 | 2.27 | 2.25 | 2.26 | 2.25 | 2.22 | 2.28 |
| **Light Pepsi** | 2.23 | 2.18 | 2.19 | 2.28 | 2.22 | 2.19 | 2.12 | 2.15 | 2.10 |
| **Regular Pepsi** | 1.80 | 1.80 | 1.81 | 1.78 | 1.85 | 1.85 | 1.80 | 1.82 | 1.80 |
| **Smirnoff Ice** | 28.30 | 29.30 | 29.30 | 28.10 | 27.38 | 28.40 | 26.65 | 27.35 | 26.80 |
| **7 Up** | 9.80 | 9.80 | 9.80 | 9.80 | 9.74 | 9.80 | 9.70 | 9.32 | 9.60 |
| **Blind** | 20.09 | 19.40 | 19.57 | - | - | - | - | - | - |

*Key for sample codes: First digit = batch; second digit = number of determination*

**Table 2: Concentration of phosphoric acid in beverage samples obtained from potentiometric titrations**

| Sample | Concentration (mg P/L) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 11 | 12 | 13 | Mean | 21 | 22 | 23 | Mean | 31 | 32 | 33 | Mean |
| **Light Coke** | 143.4 | 135.6 | 131.8 | 136.9±5.9 | 139.5 | 139.5 | 139.5 | 139.5±0.0 | 139.5 | 139.5 | 139.5 | 139.5±0.0 |
| **Regular Coke** | 182.9 | 187.6 | 179.8 | 183.4±3.9 | 175.9 | 174.4 | 175.2 | 175.2±0.8 | 174.4 | 172.1 | 176.7 | 174.4±2.3 |
| **Light Pepsi** | 172.8 | 169.0 | 169.7 | 170.5±2.1 | 176.7 | 172.1 | 169.7 | 172.8±3.6 | 164.3 | 166.6 | 162.8 | 164..6±2.0 |
| **Regular Pepsi** | 139.5 | 139.5 | 140.3 | 139.8±4.5 | 138.0 | 143.4 | 139.5 | 141.6±3.1 | 138.0 | 141.1 | 139.5 | 140.0±0.9 |
| **Smirnoff Ice** | 2193.3 | 2270.8 | 2270.8 | 2244.9±44.7 | 2177.8 | 2122.0 | 2201.0 | 2166.9±40.6 | 2064.4 | 2119.6 | 2077.0 | 2087.3±28.6 |
| **7 Up** | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| **Blind** | 1556.98 | 1503.50 | 1516.68 | 1525.7±27.9 | - | - | - | | - | - | - | |

*Key for sample codes: First digit = batch; second digit = number of determination*

## Results of spectrophotometric determinations

Table 3 presents the concentration of phosphoric acid in the beverage samples/blind and their means (as per batch).

**Table 3: Concentration of phosphoric acid in beverage samples obtained from spectrophotometric method.**

| Sample | Concentration (mg P/L) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 11 | 12 | 13 | Mean±sd | 21 | 22 | 23 | Mean±sd | 31 | 32 | 33 | Mean±sd |
| **Light Coke** | 222.8 | 227.2 | 230.1 | 226.7±3.7 | 205.3 | 207.7 | 208.7 | 207.2±1.8 | 222.3 | 225.5 | 225.9 | 224.6±2.0 |
| **Regular Coke** | 215.5 | 218.9 | 220.8 | 218.4±2.7 | 268.4 | 272.1 | 275.0 | 271.8±3.3 | 241.0 | 242.9 | 244.1 | 242.7±1.6 |
| **Light Pepsi** | 217.7 | 222.3 | 221.3 | 220.4±2.4 | 250.2 | 253.9 | 254.9 | 252.9.8±2.3 | 253.6 | 255.1 | 256.0 | 254.9±1.2 |
| **Regular Pepsi** | 235.7 | 237.8 | 238.3 | 237.3±1.4 | 254.8 | 256.8 | 257.0 | 256.2±1.2 | 253.6 | 256.0 | 257.0 | 255.6.0±1.8 |
| **Smirnoff Ice** | 890.2 | 917.3 | 940.7 | 916.1±15.3 | 959.1 | 967.9 | 980.5 | 969.1±10.7 | 901.8 | 927.1 | 940.7 | 923.2±19.7 |
| **7 Up** | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| **Blind** | 1540.6 | 1545.0 | 1549.4 | 1545.0±4.4 | - | - | - | - | - | - | - | - |

*Key for sample codes: First digit = batch; second digit = number of determination*

## Efficiency

The efficiency of the two key equipment used were calculated and found to be 98.43% and 99.68% for the pH meter and UV-Visible spectrophotometer respectively.

**Analyses**

In the analysis of variance using Student's t test to compare the results of the estimation of phosphoric acid in the beverages using potentiometric titration and spectrophotometric methods, $T_{cal}<T_{tab}$ for the three batches and the standard, at 0.05 level (that is, 0.756< 2.13; 0.651< 2.13; 0.642< 2.13; 1.19 < 2.92 for batch 1, 2, 3 and the standard/blind respectively) it can be concluded that there is no significant statistical difference between the methods. Although, none of the methods is sufficient in establishing the concentration of phosphoric acid in the beverages as the potentiometric method provides information on the identity while the spectrophotometric method being more sensitive provides information on quantification.

Since there was no significant statistical difference in the use of these methods in the estimation of phosphoric acid in the beverages, the result of spectrophotometric method were used in the analysis of variance (Kruskal–Wallis H test) to elucidate the level of consistency in the levels of phosphoric acid in the various brands of beverages under study. At 0.05 level, $H_{cal}$ was less than $H_{tab}$ for Light Coke, Regular Pepsi, Light Pepsi and Smirnoff Ice in which $H_{tab}$ was 5.99 at 2 degrees of freedom while $H_{cal}$ was 5.96, 5.49, 5.96 and 5.49 respectively. It can thus be concluded that there is no significant difference in the levels of phosphoric acid between batches in these beverages. Conversely since $H_{cal}>H_{tab}$, that is 7.20 > 5.99 at 0.05 level and 2 degrees of freedom for Regular Coke, it can be concluded that there was a significant difference in the levels of phosphoric acid in the various batches of Regular Coke.

Generally, the results of the spectrophotometric method were apparently higher than those obtained from the potentiometric titration method. This may be due to removal of some of the protons from the acid; maybe during pH correction when a base is added during production to raise the pH above 2 as manifested in Figures 2(A-F) or presence of bases [14]. The initial pH of the blind (standard phosphoric acid) was found <2 while those of the beverage samples were all > 2. Whereas the spectrophotometric method measures the total amount of phosphorus present, this might not be affected by the raising of pH. In addition to the above, any form of phosphorus present will be measured by the spectrophotometric method which accounts for its higher values. Therefore, the spectrophotometric method has greater selectivity compared to the potentiometric method.

Lozano-Calero*et al.* [20] spectrophotometrically estimated phosphorus in Crystal Pepsi® and obtained results that are similar to those presented here. In contrast, the results of this work could be compared to the results of Murphy [14] except that the results of the potentiometric titration were higher than those of the spectrophotometric method.
High prevalence of exposure and excessive consumption of phosphoric acid-containing beverages may present a public health problem. The report by Duffey*et al*. [21] showed that dietary patterns and beverage consumption are important to varying degrees, for different metabolic outcomes. The extremely low initial pH values of the beverages account for the claims of authors' of been associated with cariogenicity [12,19,22].

According to the Institute of Medicine recommendations, the recommended dietary intake (RDI) of phosphorus is as follows [23]:0 to 6 months: 100 milligrams per day (mg/day), 7 to 12 months: 275 mg/day, 1 to 3 years: 460 mg/day, 4 to 8 years: 500 mg/day, 9 to 18 years: 1,250 mg, Adults: 700 mg/day, pregnant or lactating women - younger than 18: 1,250 mg/day, older than 18: 700 mg/day.Considering the recommended dietary allowance of 700 mg P/day for adults, it becomes pertinent that consumption of one litre of Light Cokefor instance, would contribute approximately 220mg to the body's phosphorus intake for the day representing approximately 29%. This appears to be quite high considering the fact that it is present in every cell of the body and that its main food sources are the protein food groups of meat and milk, whole-grain breads, cereals, fruits and vegetables. These are common food sources that people tend to consume daily at a high rate. The consumption of one litre and above of Smirnoff Ice(about 900 mg P/L) puts one at higher chances of excessive phosphorus intake and hence the attendant consequences. Since the effect of soft drink intake was no longer significant after other risk factors such calcium, potassium and sucrose intake had been controlled, suggesting that the effect of soft drink consumption on urinary stones may be a consequence of its influence on these risk factors [24,25], modest intake of these beverages may not appear to have adverse effects [26]. Sequel to the above, the public should be made to beware of this risk factor thereby checking their daily soft drink consumption or better still, regulatory bodies should set lower limits for the maximum amount of phosphorus that should be used in compounding these beverages to take into account the consequences of excess phosphorus in the human system.

## CONCLUSION

The results of the estimation of phosphoric acid content in the beverages using potentiometric titration and spectrophotometric methods, gave no significant difference since $T_{cal}<T_{tab}$ for the three batches of the beverages under study at 0.05 levelin the analysis of variance using Student's t test, but no single method is sufficient in establishing the concentration of phosphoric acid in the beverages. Considering the level of consistency in the

phosphoric acid content in the different batches of the brands under study using the Kruskal-Wallis H test, there was no significant difference in the levels of phosphoric acid content in Light Coke, Regular Pepsi, Light Pepsi and Smirnoff Ice at 0.05 level and two degrees of freedom. Conversely, there was a significant difference in the levels of phosphoric acid within the three batches of Regular Cokeat 0.05 level. However, the extremely low pH values of the beverages(2.15 – 2.85) account for the claims of authors of been associated with cariogenicity. In addition to the above, in consideration of the recommended dietary allowance of 700 mg P/day for adults, the phosphoric acid content of these beverages (range from 207.241±1.751 – 969.147±10.743 mg P/L) poses some health concern.

**Acknowledgements**
In addition to approving the use of the facilities in the Department of Chemistry, Benue State University, Makurdi, the Head of Department, Dr S.T. Ubwaalso funded the purchase of all the samples for the laboratory analyses. The technical support from Mr G.H. Atoois gratefully acknowledged.

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