Thesis Report

On

"Comparative study and Quantitative determination of crucial heavy metals caffeine, pH, in carbonated beverages available at the local market in Bangladesh "

Submitted to

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LETTER OF TRANSMITAL

Date: 20th December 2018

Professor Dr, Md. Bellal Hossain

Head, Department of Nutrition and Food Engineering

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Subject: Submission of project report

Dear Sir,

I would like to take this opportunity to thank you for the guidance and support that you have provided me during the course of this report. Without your help, this report would have been impossible to complete.

To prepare the report I collected what I believe to be most relevant information to make my report as analytical and reliable as possible. I have concentrated my best effort to achieve the objectives of the report and experience gathered during report preparation will immeasurably help in my future professional life. I request you to excuse me for any mistake that may occur in the report despite of my best effort.

I would really appreciate it you enlighten me with your thoughts and views regarding the report.

I therefore, would like to place this report for your judgement and suggestion. Your kind advice will encourage me to perform better planning in future.

Sincerely Yours

……………………………......

Hayatun Nabi

ID: 151-34-351

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CERTIFICATE OF APPROVAL

I am pleased to certify that the internship report on "Quality Control Assurance and Production of Bakery Products" in "Well Food" conducted by Hayatun nabi bearing respectively ID NO: 151-34-351, department of Nutrition and Food Engineering has been approved for presentation, defense and viva-voce.

I am satisfied to certify that the data and the findings in this report are authentic work of Hayatun Nabi. I strongly recommended the report presented by Hayatun Nabi for further academic recommendations and defense and viva-voce Hayatun Nabi, bears a strong moral character and well personality. It has a great pleasure working with him and wish him a successful life.

………………………………………..

Professor Dr, Md. Bellal Hossain

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I am encouraged to take this privilege to deliver my gratitude to each and every people who are involved with me in every phase of my lives. I am very grateful to Professor Dr. Md. Bellal Hossain**,** Head, Department of Nutrition and Food Engineering, Daffodil International University

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ABSTRACT

This Study did to determine the pH ,level of caffeine concentration and amount/level of heavy metals concentration ,total suspended solids and total dissolve solids of five soft drinks sample that are locally available in the market of Bangladesh to ensure whether the pH level ,level of caffeine concentration and level of heavy metals concentration follow the FDA recommendations 0r not. Quantitative estimation of Caffeine and heavy metals had done by the Uv-Spectrophotometer and Atomic absorption spectroscopy ,pH were measured by the Hannah pH meter .Caffeine , pH , and heavy metals were determined with guided methods of AOAC. All the methods are established by the regulatory bodies. The minimum caffeine content was found in sample 3(85mg per 250ml drinks),where the maximum amount of caffeine was found in sample 2 (180mg per 250ml drinks). The concentration of caffeine in all energy drink samples are well below the maximum allowable limits set by the food regulatory bodies, except the sample 2. From this study , the minimum caffeine level of soft drinks were showed by sample 3 where sample 2 showed the highest caffeine level. And the pH range of these sample 2.30 to 3.00. Which is not consistent with the food regulatory bodies . Sample 2 showed lowest pH level where sample 3 showed much higher pH level ,so sample 2 is more acidic than the other sample .There is a relation between the caffeine and pH. If any soft drinks contain higher level of caffeine concentration with very low pH that stimulants human's central nervous system rapidly than the other sample . This study also evaluated some constituents of five soft drinks in Bangladesh and investigated the presence of some heavy metal contaminants. All the sample of soft drinks were screened for the presence of sugar, carbon dioxide, phosphate . The level of cadmium, and lead were determined using atomic absorption spectrophotometer. This study also showed the presence of sugar, carbon dioxide, phosphate, in the soft drink. All the samples of this study contain Lead and Cadmium. All the samples contained lead content within the actual limit except one sample that was not consistent with the standard limit that is specified with appropriate bodies . All the samples contained lead content within the range of 0.3642mg/l to 11mg /l .Only sample P contain the highest amount of Lead content (11mg/l) which was more higher than the actual limit of regulatory bodies that is 0. .The presence of cadmium in all the samples range from 0.050 mg/l to 0.112 mg/l which was within the limit of WHO limit .Sample T contained the maximum amount of cadmium (0.113mg/l) whereas Sample S contain the minimum amount of Cadmium (0.050mg/l) both are consistent with actual limit of regulatory bodies .

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

The Term " Soft drinks " is refers to the Beverages that is claim to be provide the lot of energy with combination of the caffeine and other plant based stimulants like (e.g., guarana, yerba mate),and simple sugar like glucose and fructose and also glucuronolactone that is naturally occurring glucose metabolite,it also contain the amount of Amino acid like, carnitine, taurine, creatine and herbs and vitamins as well [1]

Caffeine in known to be organic molecule that is present in the beverages like tea ,coffee , energy drinks ,and cola etc .Caffeine is very known to us because it is caffeine which stimulants our brain's central nervous system. It is Agreed that there is little risk to harm when a person consume the caffeine less than 300mg a day [2]) During the time of anxiety or stress or during the pregnancy ,The FDA recommends less than 200mg of caffeine of a day(Food Standards Agency (2008) Pregnant women advised to limit caffeine consumption.)

Caffeine provide the emerging boost[3]
. it was first invented in Europe and asia in 1960 in response to purchaser demand for a dietary supplement that would result in the increased the energy that consumer want[4]

Caffeine is also added as flavoring agent and also used for make the drinks addictive[5]) . Caffeine is bitter in taste, white crystalline xanthine alkaloid that is one kind of Caffeine that acts as a psychoactive stimulant drug and a mild diuretic Analysis of 53 products having caffeine containing ingredients. [6].. There is almost sixty plant species contain caffeine like bean(seed) of the coffee plant ,in the level of the tea bush or leaves , And in the Cola nuts (Analysis of 53 products having caffeine containing ingredients[6]
In 1981 ,A Columbian scientist , Friedrich Ferdinand was first isolated pure caffeine in the laboratory . Caffeine is the world's most widely used drugs[7]

Soft drinks are also called ready to drink bevarages,these are the sweetened water based beverages[8]. These are the frequently flavored and colored and water is the principle component that is nedded for the hydration .Soft drinks are very popular to the people of every ages specially young and aged person ,they consume soft drink to quench their thirst .when the soft drinks are manufactured ,special attention must be taken to maintain the purity to and the uniformity of the ingredients ,raw materials ,source of water and the packaging materials which are actually the source of impurities in soft drinks. The presence of metallic impurities in soft drinks can constitute the various health complications to the people who consume the this kind of metal polluted soft drinks ([9] Environmental pollution is the main reason which is responsible for the heavy metal pollution in the food chain .Lead and cadmium are the potential the two potential harmful metal that have taken considerable concern (Cabrera et al., 1995).Atmospheric contamination is caused y the excessive use of fertilizer ,chemical pesticide and sewage sludge or irrigation and residual water. These all are the causes of contamination of raw food stuffs ([10]). For this reason ,the atmosphere ,soil ,underground water and surface water are polluted ,for these reason our foods and beverages are contaminated by the heavy metals(Krejpcıo et al., 2005) .Due to the high toxicity of the heavy metals ,there is public interest to quantify the actual amount of heavy metals present in the soft drinks and other food stuffs that commercially available in the market .

This research is to investigate the exposure of caffeine and heavy metals in soft drinks in Bangladesh . This will provide information on the dietary exposure to heavy metal contaminants, and its potential health implications as well as the potential sources of contamination and recommendations for action. Research findings help to provide the evidence to mobilize the support for implementing national policies that commit to the government to reducing use of excess caffeine and heavy metal contamination of soft drinks

Hence the study is carried to determine the actual amount of caffeine and heavy metals(lead,cadmium) are present in the soft drinks that are commercially available in Bangladesh and to estimate the level of the selected heavy metals (lead and cadmium) in commercially available soft drinks [11]

1.2:Caffeine

Caffeine is chemical ingredient that is considered as a central nervous system stimulant. Caffeine is the most used drugs in the world that is consumed by the 90% of the people in the world , but mostly consume from the beverages. Caffeine is the naturally occurring substance that is mostly found in coco beans ,tea leaves ,and kola nuts etc. Caffeine's strongest effects are felt when you take consume ,it becomes more stronger for about an hour after taking it, but some effects of caffeine last 4 to 6 hours. Caffeine that causes increased neuron firing in the brain, in which which the pituitary gland perceives as an emergency and therefore causes the adrenal glands to release adrenaline. Caffeine that also increases dopamine levels in the human body , the neurotransmitter that also is affected by drugs like amphetamines and heroin.so the dose of caffeine must be lower than other drugs .more over it is used as a source of caffeine's addictive quality.

1.2:The Chemical and its Sources:

Caffeine is now addressed to be "the most widely used psychoactive drug in the world[12]

there are few studies estimate that 90% or more of this people of any country uses caffeine, it consume either through foods, beverages, or prescription and over-the-counter medicinesetc[13]

The main and the most common sources of caffeine for Americans include brewed coffee, brewed tea, typical cola drinks, milk and dark chocolate, and over-the-counter medications like "Anacin" and "Vivarin."7[14]

Caffeine that is an alkaloid, or nitrogen-containing substance,that bearing the chemical formula $C_8H_{10}N_4O_2$. 8 [15]

It belongs to the members of chemicals known as methylxanthines, which also includes the closely related chemicals members of theophylline and theobromine.9

(MAESO, N. et al. Capillary electrophoresis for caffeine and pyroglutamate determination in coffees. Study of the in vivo effect on learning and locomotor activity in mice. Journal of Pharmaceutical and Biomedical Analysis, v. 41, n. 4, p. 1095-1100, 2006. PMid:16546340)

the pure form, caffeine "occurs as odorless, white, fleecy masses, glistening needles or powder."10

[16]

with all methylxanthines, caffeine that has low solubility and is therefore often combined with a wide variety of compounds that form complexes, foe example such as the double salt sodium benzoate.

The Chemical structure of Caffeine

1.3: The properties of caffeine :

Caffeine's Molecular Formula: C8H10N4O2

Caffeine's Molecular Weight : 194.19

Caffeine's Molar mass: 194.19 g/mol

IUPAC ID: 1,3,7-Trimethylpurine-2,6-dione

Melting point of caffeine : 455°F (235°C)

Caffeine's Density: 1.23 g/cm

1.4: The Metabolism of Caffeine :

TRENDS in Endocrinology & Metabolism
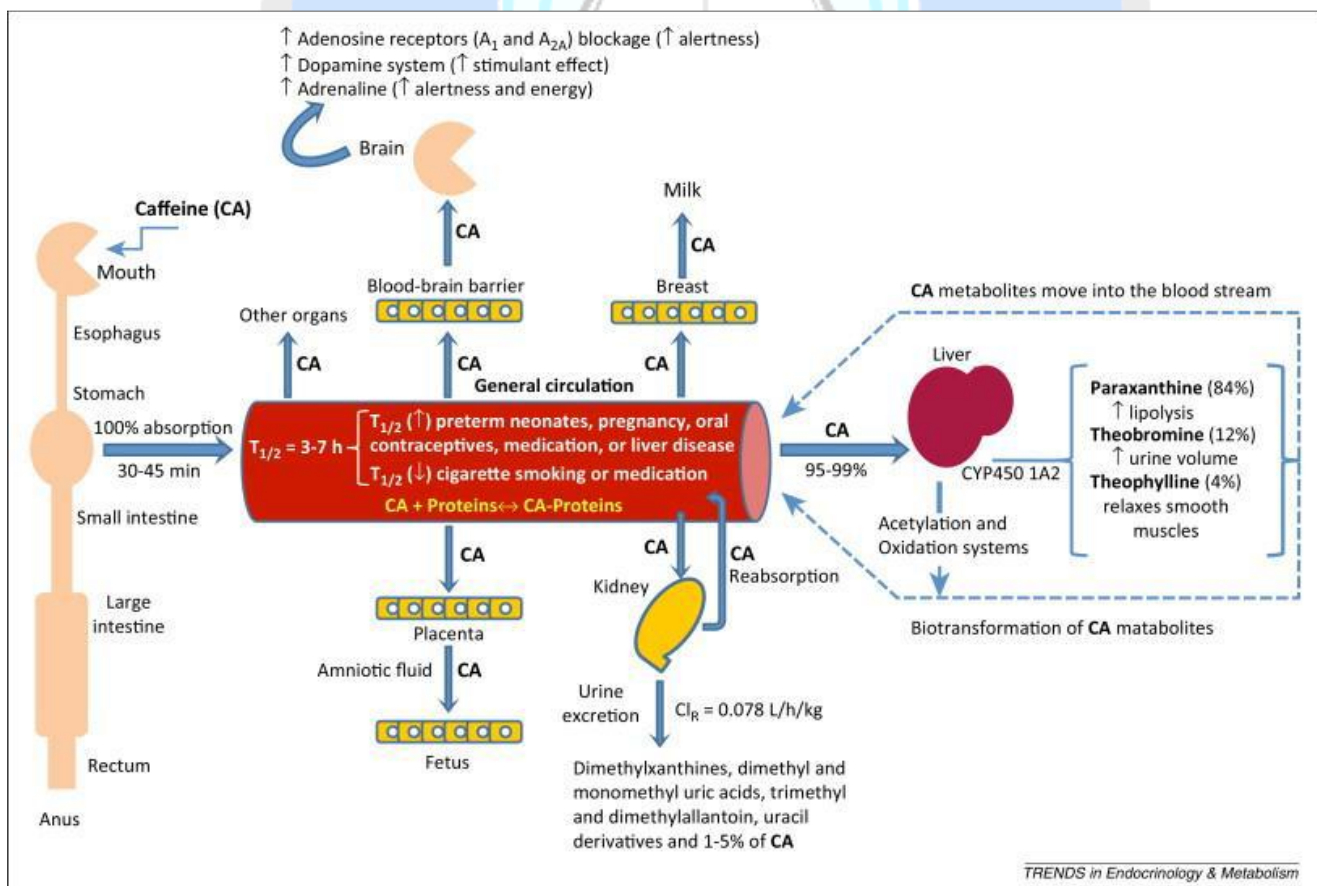
1.5 Caffeine Dosages: Quantity in Consumer Products

It is the most difficult work to set a standard dose of caffeine for the people in the world because the presence of caffeine in the various consumer goods is varying from one food to another food in every country . Some sources of caffeine suggest that one-hundred in addition to tea, cola, chocolate and over-the-counter caffeine-containing drugs."26 there is potential risk if the caffeine is taken by the adults in higher portion that it can

causes so many complications in te body ,so the adult consumption of caffeine issue   should be seriously considered more over the consumption caffeine for the adults is important, because "the potency of caffeine on a human body depends on the body's weight."27 Some sources suggest that "[t]he highest exposure to caffeine from soft drinks on a mg/ kg / day basis is among young children," especially children when they are under the age of six.

Beverage/Food	Serving Size	Caffeine
Tea	8 oz. (240 ml)	15 - 70 mg
Decaffeinated Tea	8 oz. (240 ml)	less than 12 mg
Rooibos Tea	8 oz. (240 ml)	0 mg
Herbal Tea or Tisane	8 oz. (240 ml)	0 mg
Coffee	8 oz. (240 ml)	27 - 200 mg
Decaffeinated Coffee	8 oz. (240 ml)	2 - 12 mg
Espresso	1 oz. (30 ml)	29 - 120 mg
Decaffeinated Espresso	1 oz. (30 ml)	8 mg
Chocolate (Dark)	1 oz.	20 mg
Chocolate (Milk)	1 oz.	6 mg
Pepsi MAX	12 oz.	69 mg
Mountain Dew	12 oz.	54 mg
Coca-Cola Classic	12 oz.	34 mg
7-Up and most Root Beers	12 oz.	0 mg
Rockstar Energy Drink	16 oz.	160 mg
Red Bull Energy Drink	8.4 oz.	80 mg

1.6:Mechanism of Action

Caffeine that Primarily stimulates the CNS. The Toleranc of caffeine is associated with increased adenosine receptor activity and that is shifting of A1 receptors to a high affinity state.Phosphodiesterase inhibition – this is the enzyme that is responsible for the breakdown of cAMP and therefore this action of the methylxanthines leads to increased cAMP.2nd messenger functions.Action at Ca++ channels to increase entry of Ca++ into cells and to decrease sarcolemma sequestration of Ca++. This may be related to the weak positive inotropic effect of the drug at high dose rates. Binding to GABA receptors at the benzodiazepine site[17]
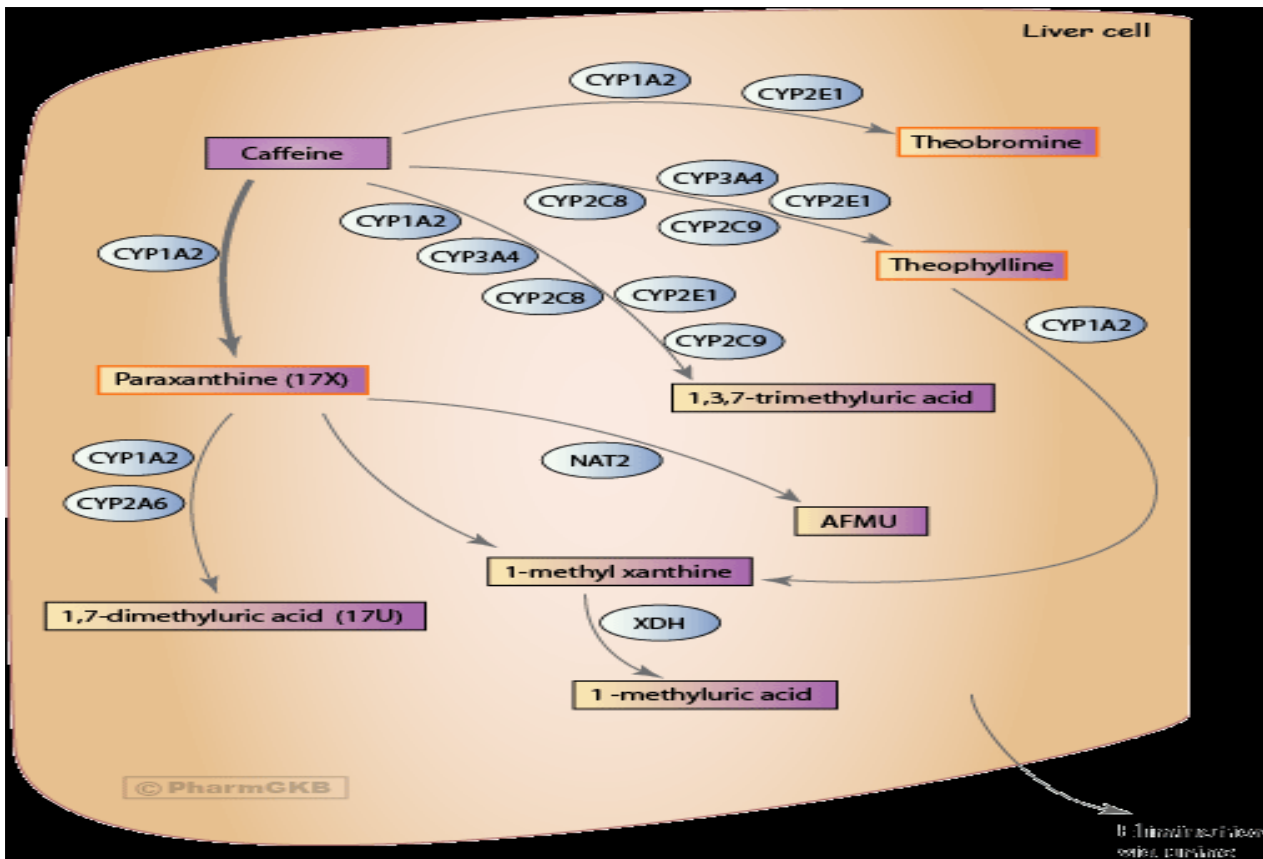
(Determination of Caffeine in Soft and Energy Drinks by HPLC ,A dissertation submitted to the Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University)

1.7: EFFECT OF CAFFEINE

1. Caffeine that mixed with carbs that replenishes muscle glycogen concentrations faster after exercise.

2. Caffeine that detoxes the liver and also cleanses the colon when taken as a caffeine enema.

3. Caffeine that helps you to keep alert while driving during periods of sleep restriction.

4. Caffeine is one of the most important factor that can stimulate hair growth on balding men and women.

5. Caffeine that relieves post-workout muscle pain by up to 48%.

6. Caffeine that relieves pain associated with sleep loss better than analgesics.

7. Caffeine that may protect against Parkinson's disease. Research shows that the man who consume coffee are at less risk of developing Parkison's disease and it even also reduces the risk of those genetically more likely to develop the condition.

8. Lower risk at cardiovascular diseases

9. Increased metabolic rate

10. Lower risk of diabetes

These all are the good side of the caffeine .people when taking caffeine through the food and drugs that is benefited health In some cases .here is some flaws of caffeine also given which causes some complications in our body because of taking caffeine for the long time .

1.8 UV spectrophotometer :

UV-Visible Spectroscopy is kind of absorption spectroscopy that has light of ultraviolet- visible region (200-700 nm.) is absorbed by the molecule. Absorption of the ultra-violet radiations happens as a results in the excitation of the electrons from the ground state to higher energy state, electrons of the molecule transfer from the lower energy ground state to higher energy state . there is law that the energy of the ultra-violet radiation that are absorbed is equal to the energy difference between the ground state and higher energy states ($\Delta E = h\nu = hc/\lambda$).

Basically , there is two types of transition one is highest occupied molecular orbital that is shoes as HUMO and the other one is Lowes unoccupied molecular orbital .the most favored transition is from the highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO). For most of the molecules, the lowest energy occupied molecular orbitals that is LUMO are s orbital, which are correspond to sigma bonds. On the other side The p orbitals are at somewhat higher energy levels, the orbitals (nonbonding orbitals) with unshared paired of electrons lie at higher energy levels.keep that in mind that The unoccupied or antibonding orbitals (π^* and σ^*) are the highest energy occupied orbitals rather than highest occupied molecular orbital

In all the compounds except (other than alkanes), the electrons undergo various transitions. Some of the important transitions with increasing energies are: n to π^*, n to σ^*, π to π^*, σ to π^* and σ to σ^*.

]

1.9: Atomic absorption spectroscopy :

Historical perspective of atomic spectroscopy : the historical background of atomic spectroscopy is closely connected to the study of the sun light .In 1802 ,the german scientist Wollaston was reported the presence of the black region in spectrum of the sunlight .after that this line have come to be known as Fraunhofer ines in honor of scientist who spent his effort illustrious carrer studying them .It was suggested ,as early as 1820 ,these Fraunhofer lines resulted from the absorption process which was occurred within the sun atmosphere .A series of studies using this very early spectrometer lead kirchoff (185(0 to suggest that any materials which can emit light a given wavelength can also absorb light at the same wavelength ,this guy was the first sciencetist to recognize that there is close relationship between the absorption spectrum and the emission spectrum of the same element .

Talbot and wheastone at 1835 reported that the colors of the flame and spark induced emission were characterstics of specific substance .this quantitative aspects of atomic spectroscopy were constructed within the last 60-70years .the development of this instruments was made possiblenot only because of ongoing progress innour understanding of the fundamental composition and behavior of atoms but also supported by the raising the realization that the presence of the minor and the trace amount of certain elements could influence industrial process appropriately .these all instrument were developed in response to a technological requirments

Modern atomic spectroscopy can be divided conveniently into the tree relted tenhniques pn the basis of the process that are used to generate then to detect and measure the free atoms of analyte . we know that AAS measure the quantity of light absorbed by the atoms of analyte ,atomic emission and atomic fluorescence measure the quantity of radiation emitted by the anaytte of the atoms which have been promoted to higher energy levels.

Since the introduction of the commercial atomic absorption spectrometer instruments in the eary 1960s .this technique has quickly gained wide acceptance t the point where surveys of intruments were available in scientific lab have suggested .moreover AAS is becoming fourth or fifth most popular instrument .

1.10 :History Of carbonated beverages (Soft drinks)

Carbonated beverages drinks is the drinks that contain carbonated water ,a sweetener and a flavoring that might be natural or artificial .The sweetener might be sugar ,high fructose corn syrup ,fruit juice or sugar substitute like aspartame ,saccharine etc . soft drinks also contain a fixed amount of caffeine ,color ,preservatives and or other ingredients.

While soft drinks are used in the product labelling or restaurant's menus , in so many country soft drinks are referred to its regional name like carbonated drinks ,cool drink ,cold drinks ,fizzy drinks ,lolly water ,pop ,seltzer ,soda ,coke, soda pop tonic and mineral .these all are the different name of soft drinks used in the different parts of the world[18]

Due to containing high sugar contain it is also called sugary drinks in many region of the world .

The origin of the soft drinks was derived from the development of the fruit flavored drinks .a variety fruit flavored drinks were widely drunk at the medieval middle east such as "Sharbat " and that were often

sweetened with ingredients such as ,sugar ,syrup and honey .also included other ingredients such as lemon ,apple ,pomogranate ,tamarind ,jujube ,sumac ,musk ,mint and ice as well .after middle estern drinks became popular in in the medieval middle Europe where the word syrup was derived from the Arabic .

In tudor England water imperial was drunk widely .it was sweetened drins mixed with the lemon flavor and containing the cream of tartar.

Another types soft drink was lemonade ,made of water and it was sweetened with the honey but without carbonated water.After a long time it is also made up with carbonated water as well . a Compagnie des Limonadiers of Paris was made a monopoly for increasing the sale of lemonade soft drinks in 1676. Vendors carried some tanks of lemonade on their backs and also dispensed cups of the soft drink to Parisians.

In 18th century ,scientist has been working for progessing or developing naturally carbonated mineral water .In 1767 an English man joseph priesetly was first discovered the infusion water with carbon dioxide to make carbonated water ,when he was suspended a bowl of distilled water above a beer vat at a local brewery in Leeds, England. His invention of carbonated water (also known as soda water) is now the major and essential component for most the soft drinks that are exist in the world .

This equipment used by Joseph Priestley in his experiments on gases and the carbonation of water at those time

Joseph found that water treated in this manner taste better rather than the normal water and then he said to his friend that carbonated is most refreshing drinks

Another Englishman, John Mervin Nooth,later improved Priestley's design and he sold his apparatus for commercial use in pharmacies. After that Swedish chemist Torbern Bergman was invented a generating instrument that was made of carbonated water from chalk by the use of sulfuric acid. Bergman's instrument allowed imitation mineral water to be produced in large scale . Swedish chemist Jöns Jacob Berzelius started to add flavors (spices, juices, and wine) to carbonated water in the late eighteenth century.

Thomas Henry, an apothecary from Manchester, he was the first scientist who sold artificial mineral water to the general public that was for medicinal purposes, very begining in the 1770s. He made his recipe for 'Bewley's Mephitic Julep' that was consisted of 3 drachms of fossil alkali to a quart of water, and also the manufacture had to 'throw in streams of fixed air until all the alkaline taste is destroyed' in this way he destroyed the taste of alkaline from the carbonated water .

This was not long time before flavoring agent was mixed with carbonated water. There was a earliest reference to carbonated ginger beer is in a Practical Treatise on Brewing. published in 1809. After some tears the drinking of natural or artificial mineral water was considered as healthy practice, and was promoted by advocates of temperance ,because consumption of the carbonated was healthy for human's health though it is also responsible some complications . at those time Pharmacists were selling mineral waters began for adding herbs and chemicals to unflavored mineral water. They literally used birch bark dandelion, sarsaparilla, fruit extracts, and other substances in carbonated mineral water .some flavorings agents were also used to improve the taste of carbonated water .

CHAPTER TWO

LITERATURE REVIEW

2.1:

Author : Md. A. Motaleb Bhuiya et al

The main aim of this study is to determine the concentration of caffeine in enegy drinks that are locally avaiable in Bangladesh to ensure the concentration of caffeine in energy drinks follow the FDA limit or not .Five samples of enerhy drinks ere collected from the local market were stuidied in this experiment . Caffeine content as analyzed and quantified of these five sample by Uv spectroscopy . the study showed that there is no significant difference between the four sample except one sample .

Division	Caffeine content (mg per bottle)				
	Tiger	Speed	Black Horse	Thunder	Power
Dhaka	33.943	21.429	54.496	38.994	37.500
Rajshahi	36.092	24.898	79.347	43.708	41.786
Chittagong	29.480	38.061	50.033	36.435	37.653
Khulna	38.957	33.776	73.837	38.320	47.092
Sylhet	31.022	28.316	71.633	38.724	35.714
Barisal	29.535	26.122	77.143	42.496	43.469
Rangpur	35.486	30.306	78.245	37.849	41.276

2.2:

Author : This study had done by one of the student of Daffodil International University ,department of Pharmacy

This study was conducted by one of the student of Daffodil international university ,the main purpose of the study was to to determine the caffeine caffeine content in some selected soft drinks named , coca-cola ,speed ,mojo ,black horse ,tiger etc .the study showed that maximum of the sample contained the caffeine percentage within the FDA limit except one or two products .other wise there was signficant difference of caffeinecontent among the sample .

Sample	Caffeine Content / Five ml
Cocacola	1.6gmg
Speed	3.21mg
Pepsi	1.57mg
Mojo	1.91mg
Tiger	1.72mg
Black Horse	3.33mg

2.3:

Author : Sohail Ahmad1, Asma Khalid2, Nadia Parveen3, Ayesha Babar4, Rida Aslam Lodhi5, Badar Rameez6, Amber Shafi5, Nadia Noor7, Faiza Nasee

This study did to determine the level of pH and caffeine concentration in the six samples that were colllected in the from the local market of pakistan . the study was conducted to ensure that all the sample's pH and caffeine were in the rage or not according to the FDA standard .the study showed that maximum of the sample contain lower pH which means most of the sample were acidic in nature ,also contain higher caffeine content in those sample ,whose pH were low .

Brands names	Con in mg/L	Con in mg/L with dilution factor	pH
Brand 1	3.762	37.620	2.45
Brand 2	1.234	12.340	3.02
Brand 3	1.069	10.690	2.57
Brand 4	1.911	19.110	2.71
Brand 5	4.217	42.170	2.29

2.4:

Author : S. Akther1* , S. M. S. Shahriar2 , J. Sultana1 and M. K. Alam1

This study was conducted by the students of Chittagong university ,this study did a with total 18 non-canned soft drinks and 12 canned soft drinks of different brands were collected from markets in Chittagong City of Bangladesh. It was observed that , lead concentrations were significantly low in non-canned soft drinks. The highest level of the lead content was observed 0.016 mgL -l . and the content of lead in non-canned soft drinks was observed in the range from 0.005 mgL -l to 0.016 mgL -l .. Among all non-canned soft drinks, chromium was present in 11 samples. The highest concentration of chromium was found 0.018 mgL -l . The concentrations of chromium in non-canned soft drinks were in the range from 0.006 mgL -l to 0.018 mgL -l

3.5:

Author : Engwa Azeh Godwilla,c,∗, Ihekwoaba Cynthia Janea, Ilo Uchenna Scholasticab,Unaegbu Marcellusa, Ayuk L. Eugeneb, Osuji Amarachukwu Gloriaba

This Study did to determine the pH ,level of caffeine concentration and amount/level of heavy metals concentration ,total suspended solids and total dissolve solids of soft drinks sample that are locally available in the market of Nigeria to ensure whether the pH level ,level of caffeine concentration and level of heavy metals concentration follow the FDA recommendations r not. Quantitative estimation of Caffeine and heavy metals had done by the Uv-Spectrophotometer and Atomic absorption spectroscopy ,pH were measured by the Hannah pH meter.

The study showed that soft drinks were acidic in nature, pH ranging from 3 to 5 with a mean of 3.6 and . Lead was observed in all the samples ranging from 0.17 to 3.39 mg/L and it also observed mercury in22 samples ranging from 0.29 to 11.32 mg/L whereas cadmium waspresent only in one sample (0.149 mg/L).

CHAPTER THREE

PURPOSE OF THE

STUDY

3.1 PURPOSE

The use of energy beverages is high among the general population and military personnel. The main purposes of this study are given below:

- Most of the soft drinks and energy drinks available in Bangladesh contain caffeine in significant amount. But they don't mention the actual amount or sometimes don't contain the caffeine in the label. Our aim is to find out which sample contain the caffeine compound and in what amount.
- Second purpose is to determine the actual amount of caffeine compund in each bottle or per ml.
- To Determine the Actual level pH ,Actual Amount Of TSS,TDS,and TS
- Are these sample of soft drinks and energy drinks consumption harmful for our body is the another aim of this study.
- To Find the Actual Amount of heavy metals present in these samples
- This study had by the mainly two instrument one is UV-Spectrophotometer and Atomic Absorption Spectrophotometer

CHAPTER FOUR

METHODOLOGY
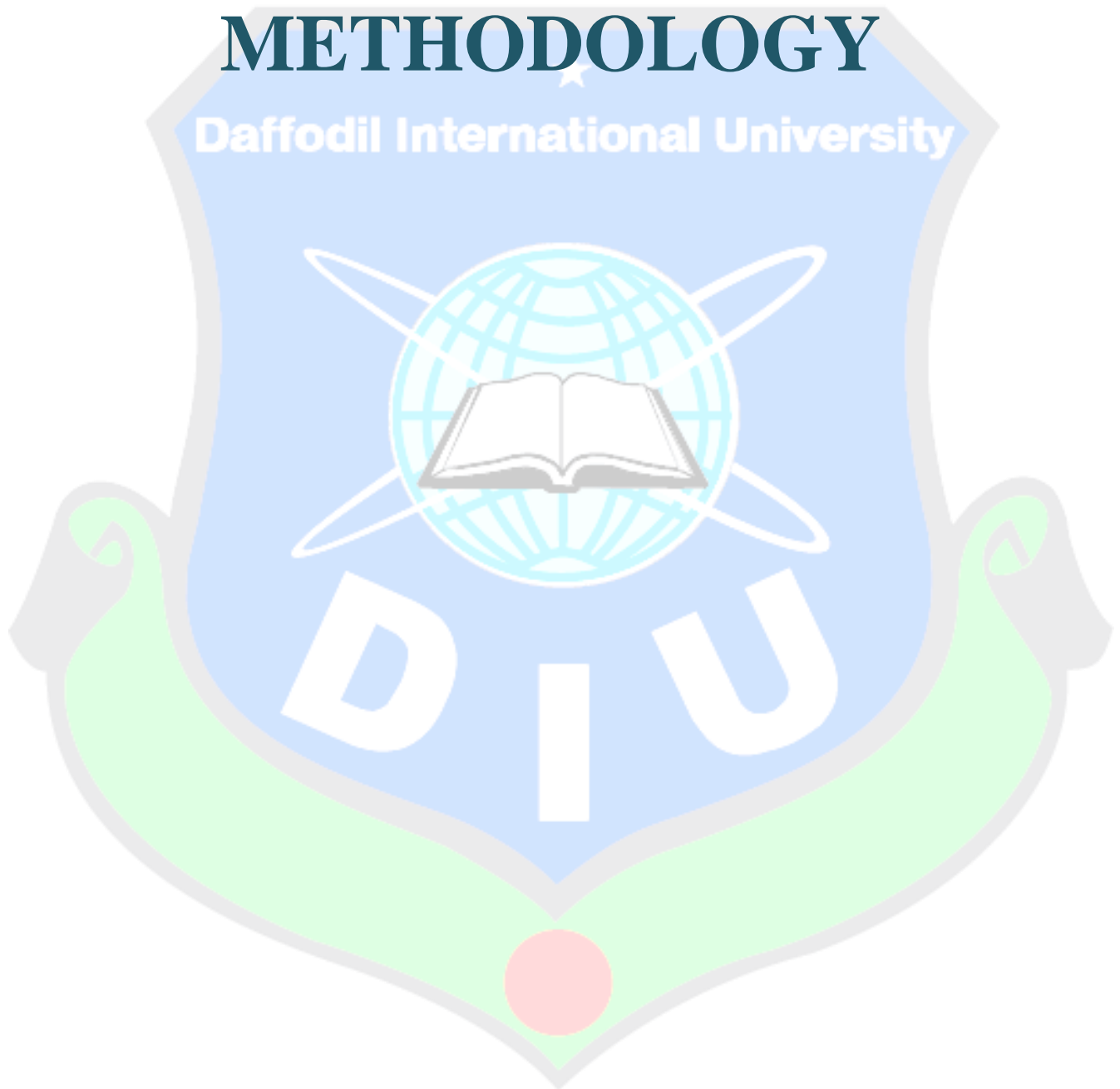
MATERIALS AND METHODS

4A:Table : Reagent and solvent details of study

SI. No	Name	Source	Country of Origin
1	Deionized water for AAS	Local Market	Bangladesh
2	Activated charcoal	Local Market	Bangladesh
3	Cocacola	Local Market	Bangladesh
4	Speed	Local Market	Bangladesh
5	Mojo	Local Market	Bangladesh
6	Pepsi	Local Market	Bangladesh
7	Tiger	Local Market	Bangladesh

4B:Table : Instrumental details of study

SI. No	Name	Source	Country of Origin
1	UV-visible Spectrophotometer	T-80 9(double beam)	Korea
2	Atomic Absorption Spectrophotometer	AA700,Shimadzu corporation	Japan
3	pH Meter	Hannah corporation	UK
4	Analytical Balance	OHAUS	China
5	Dryer	JS RESEARCH INC.	South Korea

4.1:Qualitative Test

Five sample of soft drinks name Pepsi,Coa-cola,Mojo,Tiger and Speed are taken from local market of Bangladesh for the qualitative analysis like PH,Acidity ,Sugar , carbon-dioxide ,Phosphate ,Reducing sugar ,etc . The presence of sugar, carbon dioxide, phosphatesand acidity can be determined by using this procedure.

4.1.1:Test for sugar:

Material :1.test tube,2.beaker 3. Water bath .4,benedict solution 5.sample etc

Procedure :

Benedict solution Preparation
↓
3ml of different sample were taken in different test tube
↓
Add 2ml of Benedict reagent in each of the sample's test tube
↓
The test tube is then heated in water bath for five minutes
↓
Formation of reddish color confirmed the presence of sugar

4.1..2 Test for reducing sugar:

Material :1.test tube,2.beaker 3. Water bath .4, Fehling's solution 5.sample etc

Procedure :

Fehling solution preparation

↓
3ml of different sample were taken in different test tube
↓
Add 2ml of Fehling's reagent in each of the sample's test tube
↓
The test tube is then heated in water bath for 10 minutes
↓
Formation of the brown precipitation confirmed the presence the Reducing Sugar

4.1.3Test for phosphates:

Material :1.test tube,2.beaker 3. Water bath .4, ammonium molybdate 5.sample 6.Concentrated nitric acid .

Procedure

3ml of different sample were taken in different test tube

↓

Add ammonium molybdate followed by 2 ml of concentrated nitric acid (HNO_3)

↓

The test tube is then heated in water bath for 10 minutes

↓

Formation of the yellow precipitation confirm the presence the phosphate ion

4.1.4 Test for carbon dioxide:

Material :1.test tube,2.beaker 3. Water bath .4,calcium hydroxide 5.sample 6.Lime water

Procedure :

3ml of different sample were taken in different test tube

↓

Add 2ml of lime water of (calcium hydroxide) in each of the sample's test tube

↓

Change of lime water from colorless to milky that confirm the presence of carbon dioxide

4.2 Quantitative test :

a.Total Dissolve Solid(TDS): the term dissolve solid refers to the materials like minerals , salts ,metal ,cations and anion etc that are dissolve in the water .And TDS that means Total dissolve solid that is composed of inorganic salt like calcium ,magnesium , potassium , sodium etc .and small amount of `organic matter that are dissolve in the water [19]

b. Total suspended solids (TSS) : TSS is the suspended particles that are dried .that are not dissolve in sample .It can be trapped by the filter that is analyzed by using filtration instrument of material .it is addressed as the water quality parameters that is used to assess the quality of a specimen of any type of water or water body [23]

c.Total Solids: Total solids is the amount of the total suspended and dissolved solids in water. Suspended solids are those which can be present on a water filter and that are capable of settling out of the water column onto the stream bottom when stream velocities are low [24]

Procedure of determining TDS,TSS,TS:
4.2.1 For TSS:

Materials : 1.Funnel 2.conucal flak , 3.wattman filterpaper 4. Oven . 5,weighing balance

Procedure :   Taking some funnel ,conical flask, and whatman filter paper

Clean all the required apparatus

Then weighing the weight of filter paper

After that placing the funnel above the conical flask

Place the filter paper into the funnel

↓

Then measure the soft drinks by pipette or measuring cylender

↓

Then put the drinks into the flask through the filter paper

↓

After that let drinks to store into the bottom of the conical flask

↓

After almost 3-4hours (depends on the product) remove the filter paper from the funnel

↓

And put the filter paper into the oven until the filter paper getting dry

↓

Then placethe filter paper into the dessicator for 30mints for removing the rest of the moisture that present even after drying

↓

Finally weight the filter paper

↓

Calculation

Calculating total suspended solids (TSS) concentration:

$$\frac{A-B \times 100}{C}$$

A=dried Sample weight + filter paper weight(mg)

B= Filter paper weight before drying (mg)

C= volume of the sample(ml)

4.2.2 :For TDS:

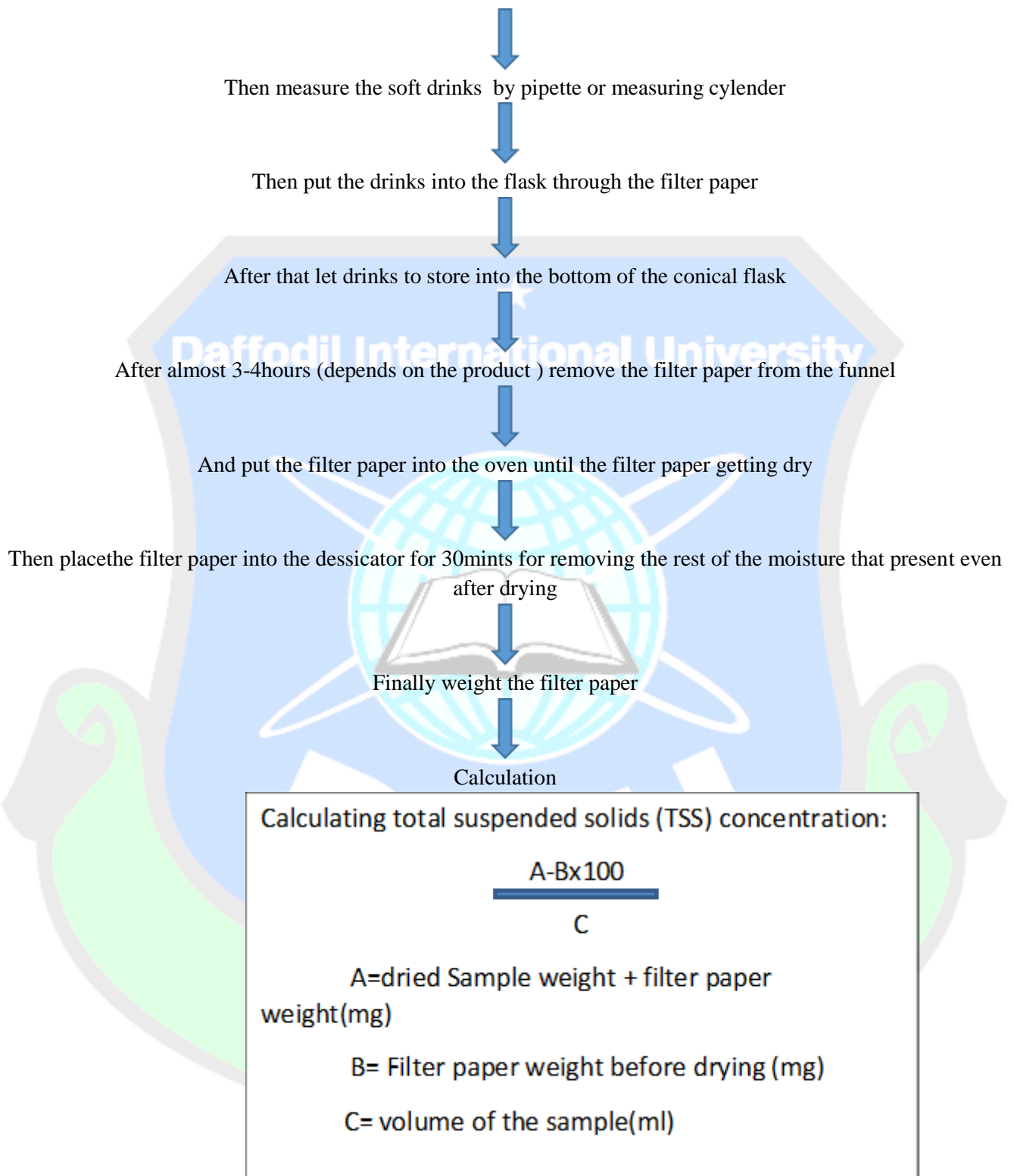Materials :1.Funnel 2.conucal flak , 3.wattman filterpaper 4. Oven . 5,weighing balance 6.sample etc

Procedure : Taking some funnel ,conical flask, and whatman filter paper

↓

Clean all the required apparatus

↓

Then weighing the weight of filter paper and conical flask

↓

After that placing the funnel above the conical flask

↓

Place the filter paper into the funnel

↓

Then measure the soft drinks by pipette or measuring cylender

↓

Then put the drinks into the flask through the filter paper

↓

After that let drinks to store into the bottom of the conical flask

↓

After almost 3-4hours (depends on the product) remove the filter paper from the funnel

↓

And Place the conical flask into the drying oven where all the drinks are stored for drying

↓

After drying place the flask into desiccator for at least 30minutes to remove the rest of the water after drying

↓

Then weighing the conical flask after drying

↓

Calculation

Calculation for TDS:

$$\frac{A-B \times 100}{C}$$

A= Weight of dried sample+conical flask weigh (mg)

B=weight of the conical flask weigh (mg

4.2.3 Total Solid: is the measure of the total amount of the suspended solid particles and dissolve solid that are retrained in the water or in other liquid sample

TOTAL SOLID(TS)= TOTAL SUSPENDED SOLID+ TOTAL DISSOLVES SOLID

4.2.4 **pH Determination :**

Ph :ph is a figure that is need to identify or specify the acidity or alkalinity of a solution is logarithm scale on which 7 is the termed as neutral and lower value indicates the acidity and higher value indicates the alkalinity .ph is equal to log10c ,where c the hydrogen ion concentration in moles/liter .more precisely it is the negative of the base of 10logarithm of the activity of hydrogen ion concentration Bates, Roger [24]

At 25c a solution with ph less than 7 is indicated acidic solution ,and a solution with ph higher than 7 is indicated as a alkaline solution .and a solution like water contain ph 7 is termed as neutral .the range of ph meters is from 0 to 14.where 0 is the strong acid indicator and and 14 is the strong alkaline indicator and 7 is the

neutral [25]

Ph measurement is very important and needed for some industrial sector like agronomy ,chemist ,food and beverage industry ,medicine and water treatment plant and on so many places .

The idea of pH was first introduced by the Danish chemist Søren Peder Lauritz Sørensen at the Carlsberg Laboratory in 1909 *[26]* Two other publications appeared in 1909 one in French and one in Danishand it was revised to the modern pH in 1924 to accommodate definitions and also measurements in terms of electrochemical cells and also for different purpose . In the very first papers, there are notation that had the "H" as a subscript to the lowercase "p", as so: pH. [27]

The real meaning of the "p" in "pH" is disputed, but according to the Carlsberg Foundation, pH stands for "power of hydrogen". It has also been suggested that the "p" stands for the German Potenz that mean (meaning "power"), also others refer to French puissance (also meaning "power", based on the fact that the Carlsberg Laboratory was French-speaking).

Materials :1. pH meter (Hannah pH Meter)

The way to measure the ph:

All the sample must be kept in a beaker or pot

Then make the buffer solution of ph 4 ,ph 7 and ph10 for calibration

After that bring the HANNA ph meter

Set the ph meter and sensor of a ph meter must dipped into the ph 4 and ph 7 buffer solution for calibration that ensure us that our ph meter works properly which increases our data's accuracy

After calibration we place the sensor of ph meter into each sample

Record the data of each sample which indicates sample's acid or alkaline property

Finally we get that data which helps to know about the property of a sample

4.2.5: Brix Determination :

Materials : 1.Refractometer 2.Water

Procedure : 1.Take the refractometer for the brix determination

2.Then wash or rinse the sensor of the refractometer for the deneutrilize the meter

3.after that take a dropper and took 1 drop of sample through the dropper

4.Put the sample into the refractometer's sensor

5.And see the degree of brix into inside scale of the refractometer .

4.3:Determination of caffeine in soft drinks that are available in local market here in Bangladesh by UV-spectrophotometer

Materials :Caffeine sample that were collected from department of nutrition and food engineering ,Daffodil international university .Activated charcoal were collected from the Hathkhola ,tikatuli .all the drinks were collected from the local market here in Bangladesh [20]
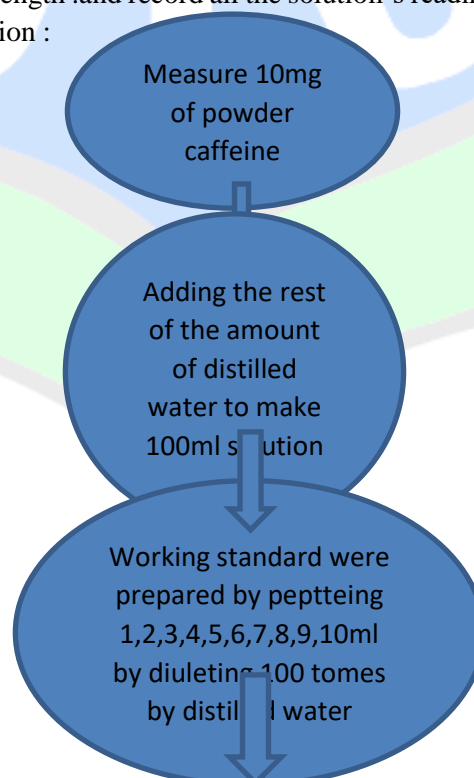
Uv-spectrophometer ,

Electronic balance,

volumetric flask ,

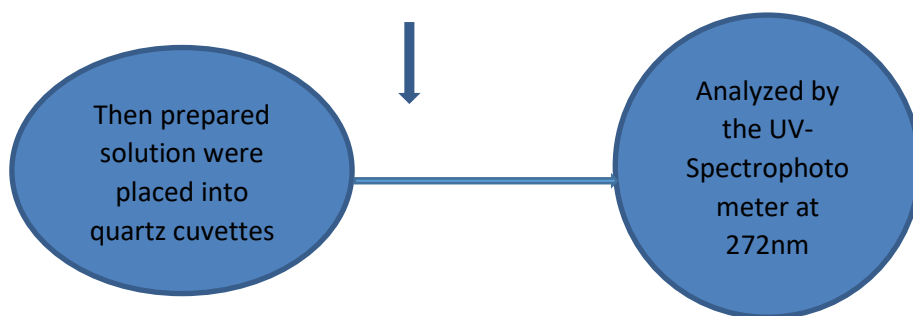measuring cylinder ,

pipette ,

Quartz cuvettes

Some beaker etc

Methodology

Sample collection : All the sample are were collected from the local market in Bangladesh ,I choose the product name ,speed ,mojo , tiger ,coca-cola , pepsi that were collated from the shop from the local market in Bangladesh[20].

Standard Preparation :100 ml of stock standard of caffeine was made by dissolving 10mg of caffeine in 100ml distilled water,after that working standards were prepared by pipetting 1,2,3,4,5,6,7,8,9,and 10ml of aliquots stock solution were separated in 100ml volumetric flask and 100 times diluted by the distilled water like 1ml of stock standard solution of caffeine added or mixed with 99ml of distilled water for making 1ml of aliquots stock solution .after that all the aliquots of the standard were placed into the quartz cuvettes and then analyze the solution by using spectrophotometer at 272nm wave length .and record all the solution's reading to make standard curve [20]

Flowchart of preparing the standard solution :

Measure 10mg of powder caffeine

Adding the rest of the amount of distilled water to make 100ml solution

Working standard were prepared by peptteing 1,2,3,4,5,6,7,8,9,10ml by diuleting 100 tomes by distilled water

Concentration (micro-gram)	Absorbance (nm)
0	0
1	0.122
2	0.235
3	0.346
4	0.473
5	0.66
6	0.732
7	0.817
8	0.942
9	0.972

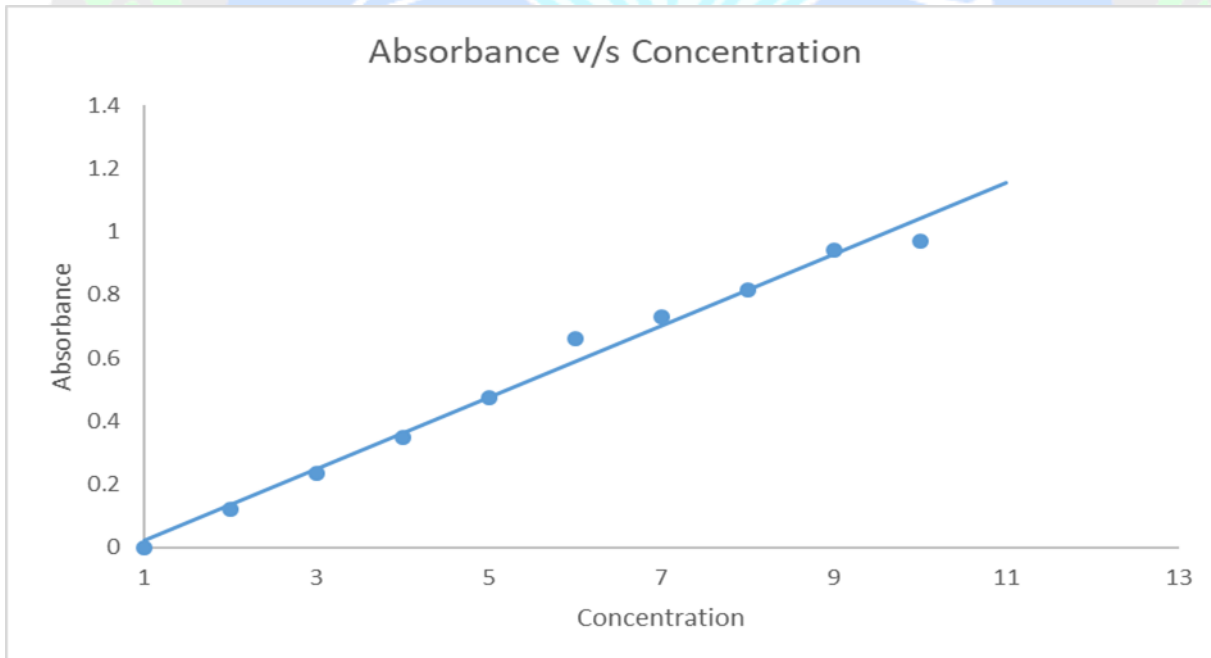**Table :Absorbance of standard preparation of active caffeine**

Figure : Calibration curve of caffeine

Calculation : From the graph we can find Calibration equation .

We know, Calibration equation ,Y=mX+C
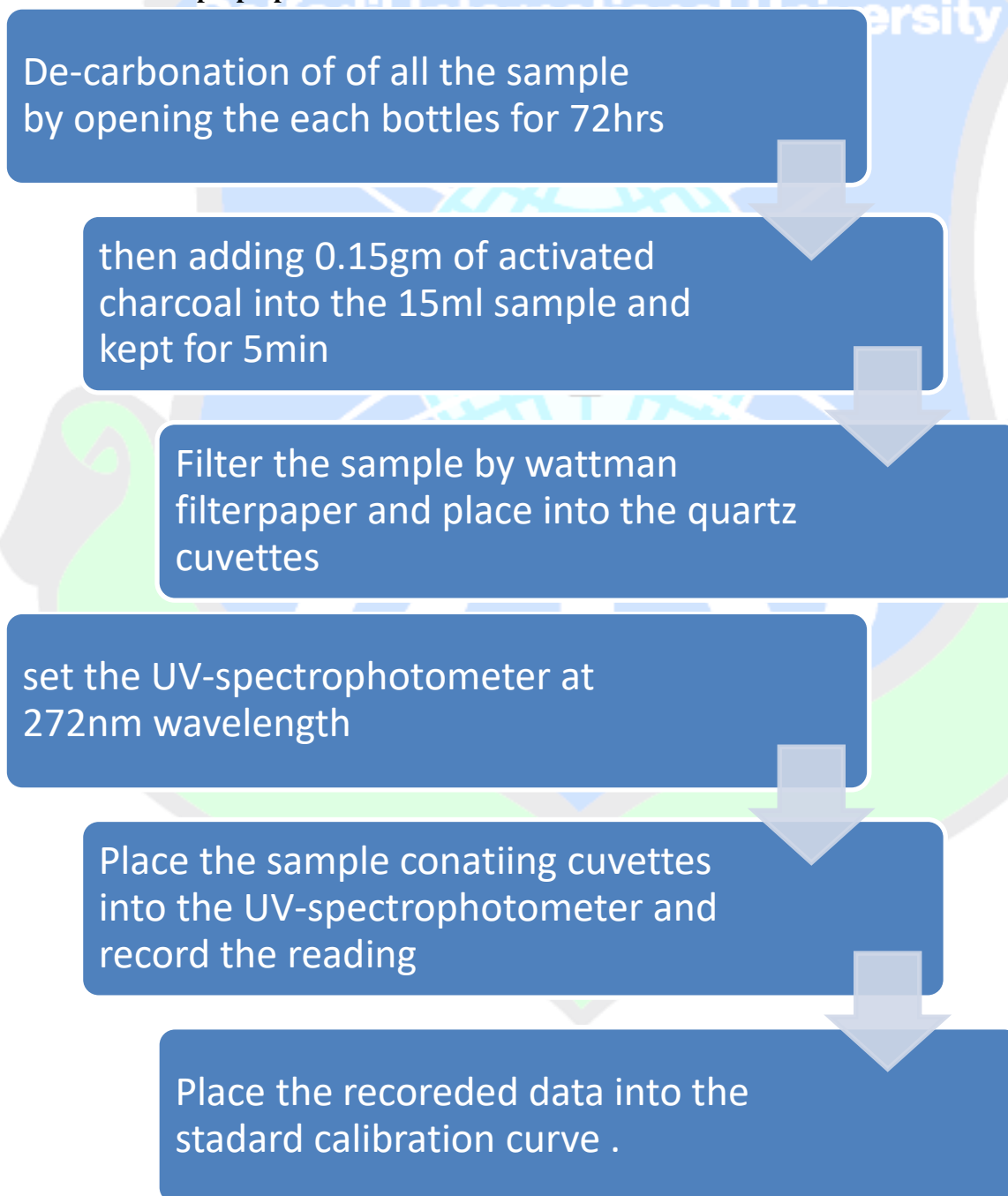
Where, "m" is the slop of the graph .

      "C" is the Yintercept .

We know that slop ,

$$m = \frac{Y2 - Y1}{X2 - X1}$$

Sample Preparation : First thing first De-Carbonation of the all sample . Each bottles of the sample was opened for 72 hours so that all the carbon di0oxide gas can go out from the bottle of the sample .then the de-colorization of the sample was run by the addition of 0.150g, of activated charcoal to the 15ml of sample ad kept the each sample for 5-10minutes .After 5 minutes ,all the samples are prepared by wattman filter paper so that all the activated charcoal are removed by the filter paper .Finally all the aliquots of filtered sample were placed into the quartz cuvettes and then analyze all the sample by using UV-spectrophotometer at 272 nm wavelength [20].

Flow chart of sample preparation :

De-carbonation of of all the sample by opening the each bottles for 72hrs

then adding 0.15gm of activated charcoal into the 15ml sample and kept for 5min

Filter the sample by wattman filterpaper and place into the quartz cuvettes

set the UV-spectrophotometer at 272nm wavelength

Place the sample conatiing cuvettes into the UV-spectrophotometer and record the reading

Place the recoreded data into the stadard calibration curve .

Standard curve preparation : The contents of the caffeine of the sample were calculated by using the peak areas

reported by the integrator and standard curve .

4.4 Quantification Heavy Metals by AAS:

Materials : All the sample were collected from the local market in Bangladesh ,cadmium and lead solution was given by Wazed mia research center ,Jahangirnagar University ,
Conical flask
Some beaker
Volumetric flask
Heating mantle
65% Nitric acid
Measuring cylinder
Pipettes
Vial
Atomic Absorption spectroscopy

Methodology

Sample preparation :
All the samples were digested according to wallace methods .In this methods 10 ml of 65% concentrated nitric acid was added ti the 25ml of sample within a heating mantle .then the mixture was evaporate on the heating mantle .Heat would continue until the all the brown fumes went out from the flask or brown fumes disappears and white fumes inside the flask .then added 50ml Deionized water into flask then the solution was concentrated by the evaporation on heat mantle to 25ml .after that filtered the solution through the glasswool and wattman filter paper .And deionized water to make 50ml solution .Deionized water also filtered through the glasswool and the wattman filter paper.[21]
Flow chart of sample preparation :

measure 10ml of65% nitric acid and added with 25ml of sample

Solution heating on heat mantle untill teh brown fumes disappears

adding 50ml water and make it concentrate by heating to 25ml

Filter the solution through the glasswool and wattman filter paper

then again adding deionized water make 50ml solution

Ready for atomic absorption spectroscopy analysis

Standard Preparation : This calibration plot methodwas described in the BritishPharmacopoeia [22] that was adopted for the making of metal ion and AAS analysis.

A stock solution of 1000ppm of the metal was made by the dividing the molar mass of the compound containing the element by the molar mass of the element .

1g of the metal ion was obtained by the equivalent weight. This 1.g of metal was dissolve with the 1000ml of distilled or deionized water to prepzare 1000ppm stock solution .then a working stock solution of 100ppm was prepared from the stock solution and serial dilution wew made from the solution that prepared for the work . The absorbance of these solutions was obtained by using AAS at 228.8, 283.3 and253.7 nm wavelength for cadmium, lead and mercury respectively.

For example:

Preparation of 20ppm stock solution of cadmium by adding 2ml cadmium solution with adding upto 100ml deionized water to make 20ppm cadmium metal standard solution .

After that from this cadmium solution ,doing serial dilution for making 100,80,60,40,25,10,0 .

There is a formulae for making serial stock solution that is ,
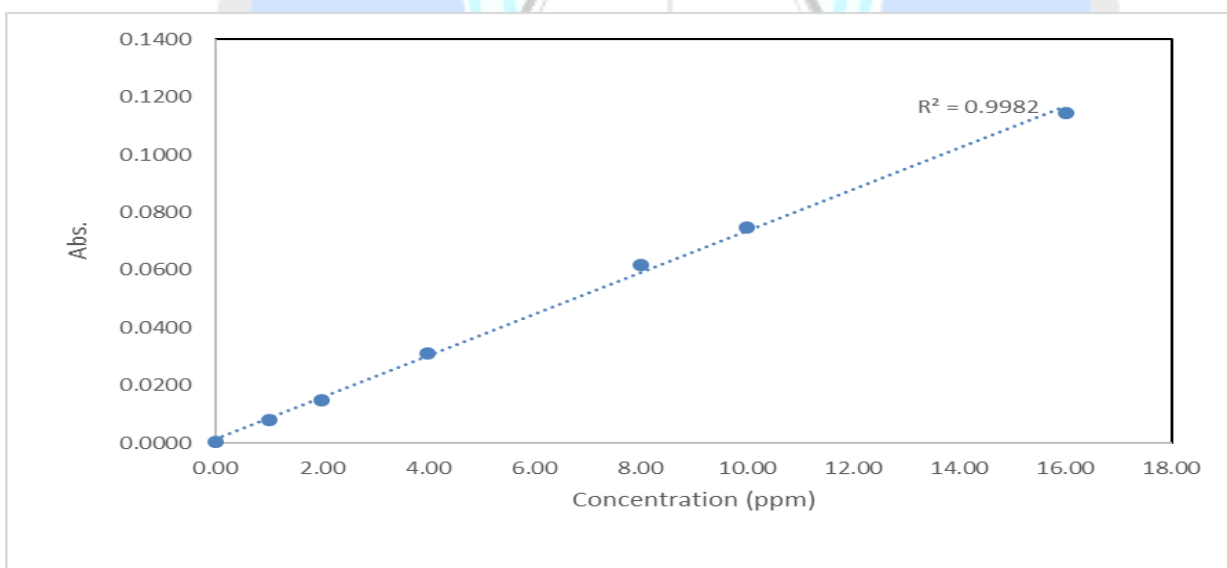
S1V1=S2V2

By using this formulae we did our rest of the serial dilution from our working solutions

Same as for the other metals like lead ,mercury ,cobalt ,chromium etc
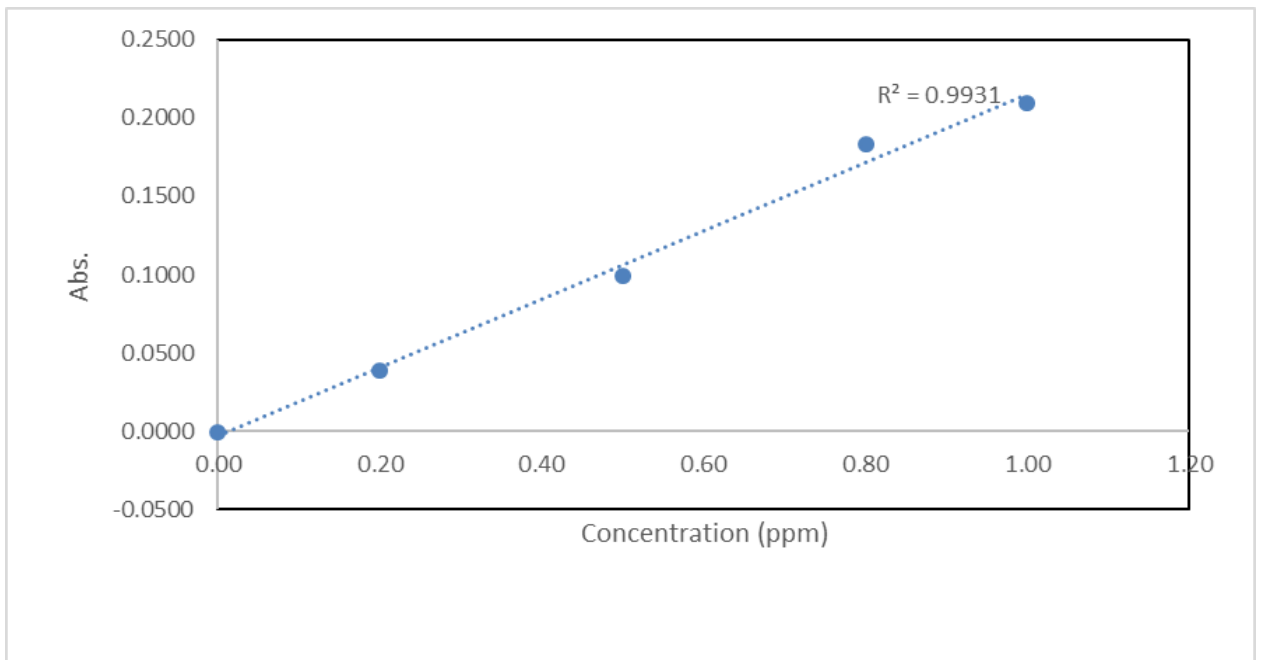
Absorbance of Pure Lead metals :

Serial No	Conc of Standard (ppm)	Abs
1	0.00	0.0004
2	1.00	0.0079
3	2.00	0.0147
4	4.00	0.0310
5	8.00	0.0619
6	10.00	0.0748
7	16.00	0.1144

Standard curve for Lead determination

Absorbance of pure Cadmium :

Serial No	Conc.of standard (ppm)	Abs
1	0.00	-0.0005
2	0.20	0.0385
3	0.50	0.0996
4	0.80	0.1835
5	1.00	0.2096

Standard curve for cadmium determination

CHAPTER FIVE

RESULT & DISCUSSION

5. RESULT and Discussion :

5.1Qualitative test

The presence of sugar, phosphate, and carbon dioxide in soft drinks

5.1.1: Presence of sugar:

Sample	Sugar
Pepsi	Present
Coca-cola	Present
Mojo	Present
Speed	Present
Tiger	Present

As all the soft drinks sample are sweet to taste ,that means all the samples of soft drinks contain sugar content. That `was confirmed as all the sample showed positive test in benedict solution test

5.1.2:Presence of Reducing sugar :

Sample	Reducing sugar
Pepsi	Present
Coca-cola	ND
Mojo	ND
Speed	Present
Tiger	Present

three of these sample out of five sample showed positive test in fehling's test which indicated the presence of reducing sugar were present. Two of these sample didn't contain reducing sugar

4.1.3: Presence Of Phosphate

Sample	Phosphate
Pepsi	Present
Coca-cola	Present
Mojo	ND
Speed	ND
Tiger	Present

,.Phosphate was observed in three samples out of five sample ,two samples didn't show any positive result in phosphate test except two samples out of five samples

5.1.4: Presence Of Carbon-dioxide

Sample	Carbon di-oxide
Pepsi	Present
Coca-cola	Present
Mojo	Present
Speed	Present
Tiger	Present

. As all the samples are carbonated soft drinks so all the sample showed positive test in carbon dioxide test ,that means carbon dioxide were present in all sample

5.2Quantitative Test :

5.2.1:The result of Total Suspended solids

Sample No	SAMPLE NAME	SAMPLE VOLUME IN ml	Final weight (g)	Iniitial weight(G)	Concentration(Mg/l)
1	Tiger	30ml	1.861	1.351	0.0567mg/l
2	Coca-cola	30ml	1.789	1.483	0.034mg/l
3	Mojo	30	1.52	1.27	0.027mg/l
4	Pepsi	30	1.54	1.29	0.028mg/l
5	speed	30	1.928	1.418	0.0566mg/l
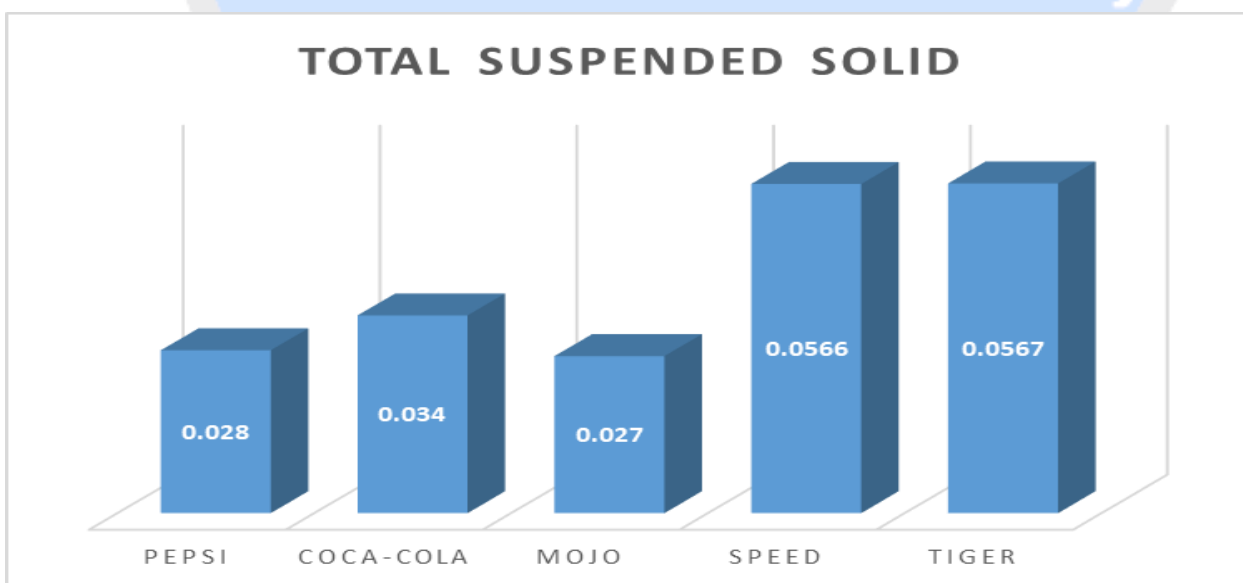
Total dissolved solid ,total suspended solid and total solid thses three factors are important for any kind drinks .because by these three factors ,able to find how much dissolve solid ,suspended solids are present in the sample and finally total solid as well .from this study ,able to find that 2of these sample out of five sample contain higerTSS, and TDS rather than other three samples . Sample S and Sample T contain the higher amount of total suspended solid than the other three samples

5.2.2Result of The total Dissolve solid :

| Sample No	Sample name	Sample volume	Final weight(g)	Initial weight (g)	Concentration (mg/l)
1	Tiger	30ml	59.126	53.156	0.663mg/l
2	Speed	30ml	58.788	52.706	0.675mg/l
3	Mojo	30ml	56.865	53.432	0.39mg/l
4	Coca-cola	30ml	56.667	53.374	0.366mg/l
5	Pepsi	30ml	59.490	56.203	0.366mg/l
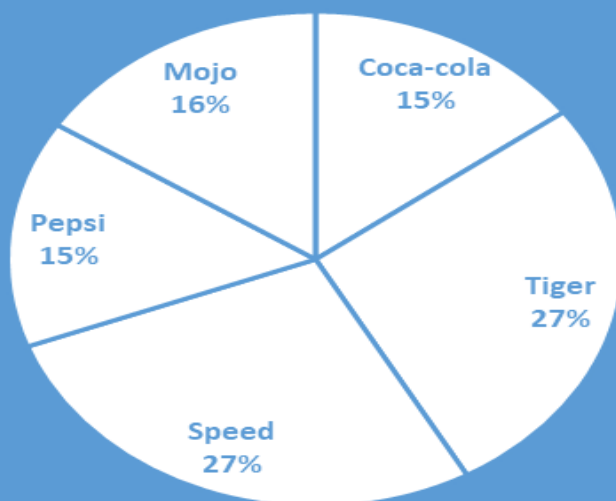
Total dissolved solid ,total suspended solid and total solid thses three factors are important for any kind liquid or semi liquid drinks .because by these three factors ,able to find how much dissolve solid ,suspended solids are present in the sample and finally total solid as well .from this study ,able to find that 2of these sample out of five sample contain higerTSS, and TDS rather than other three samples . as two of the sample out five samples also contain the higher amount of dissolve solid these two samples were sample S and Sample T rather than other sample

5.2.3:Result of Total Solids:

Sample no	Sample Name	Sample volume	TSS	TDS	Total solid
1	Tiger	30ml	0.0567	0.663	0.7197
2	Coca-cola	30ml	0.034	0.0366	0.0706
3	mojo	30ml	0.027	0.39	0.417
4	pepsi	30ml	0.028	0.366	0.395
5	speed	30ml	0.0566	0.0675	0.1242
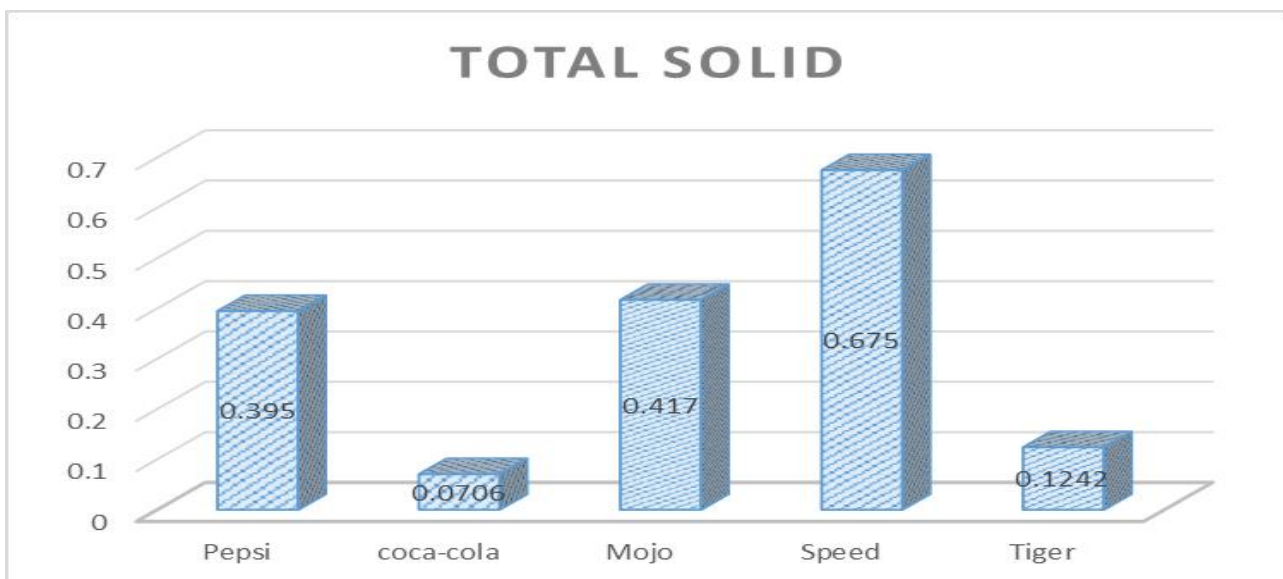
TOTAL SOLID

Total solid particles were highly present in the Sample S that is 0.1242mg/l which was quite high than other samples out of five samples .


5.2.4Result of pH of the all sample :

Sample	Average value of the ph
Pepsi	2.43
Coca-cola	2.45
Speed	2.30
Tiger	2.35
Mojo	3.00
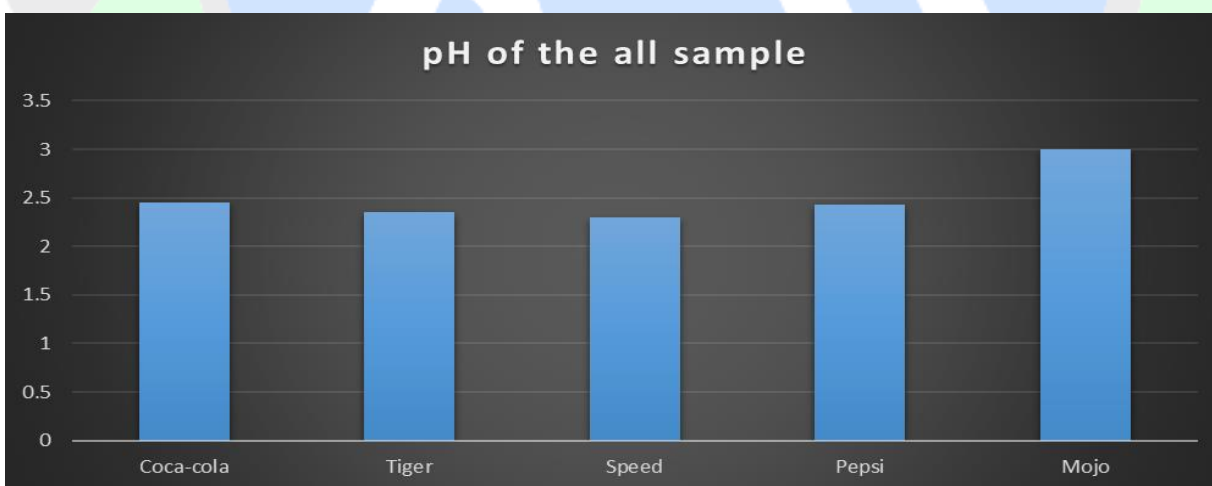
pH which is an another important factor for any kind of drinks because we know that the less the pH of any drinks the more acidic the drinks .if any drinks like soft drinks is acidic ,consuming this kind of drinks is very harmful for our body ,because acidic make so many complications in our body like it decays the our tooth ,it makes weak our central nervous system collaborative with caffeine if any one consume this kind of acidic soft drinks for log time .in this study ,able to find that five of these sample's average pH was 3 or less than 3 which indicates that all the sample are highly acidic .none of these sample's pH was in standard range that cut off value is 5.5 to 6.5

5.2.5:Brix Of the all sample :

Sample	Degree of sweetness
Tiger	16
Coca-cola	10
mojo	9
pepsi	10
speed	13
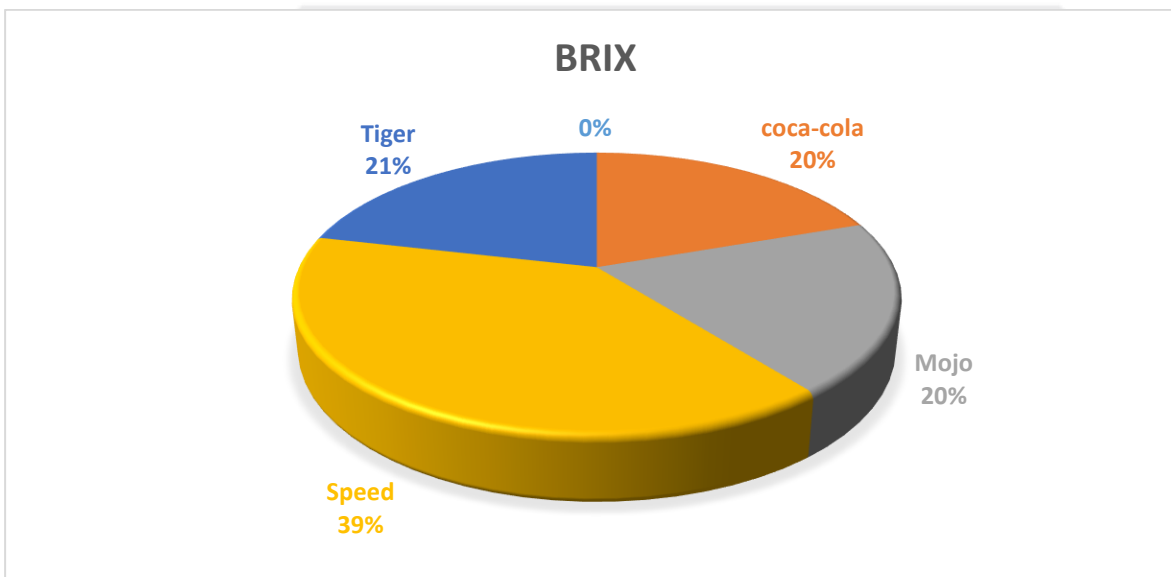
Measuring Brix is the way to know the degree of sweetness of the sample .as every soft drinks contain sugar or reducing or non-reducing sugar ,so it is important to know the degree of sweetness of the product .from this study ,able to find that al most all the sample maintain the brix level according the food and drugs administration standard level .that is 10-15 degree of brix for the drinks
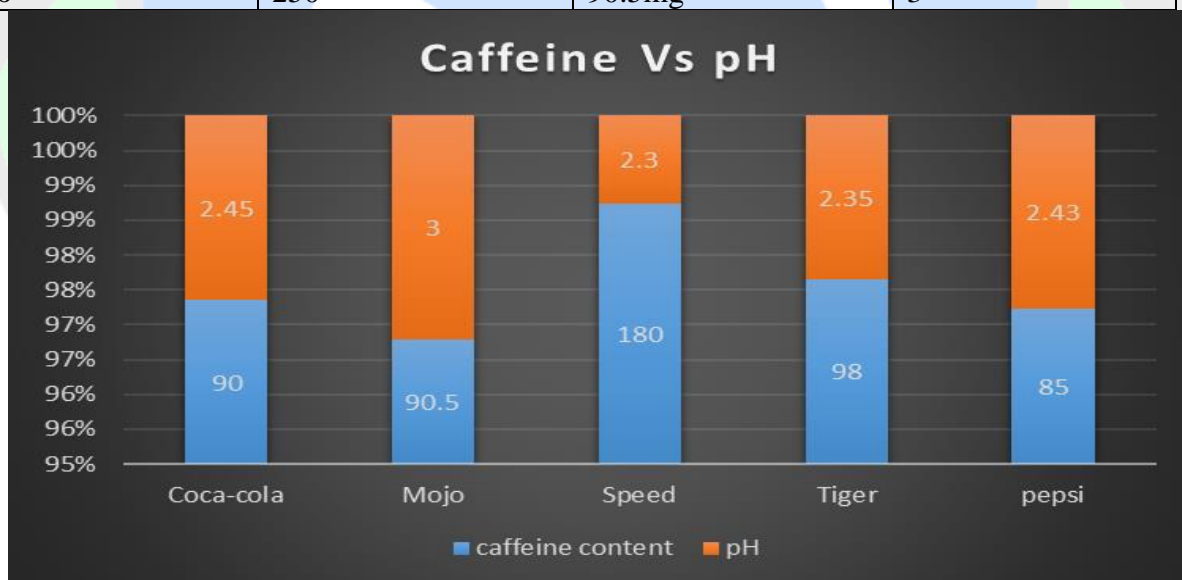
5.2.6:Caffeine :

Sample No.	Brand name	Serving size	Caffeine content mg/l
1	Coca-cola	250ml	90mg
2	speed	250ml	180mg
3	pepsi	250ml	85mg
4	Tiger	250ml	98mg
5	Mojo	250	90.5mg
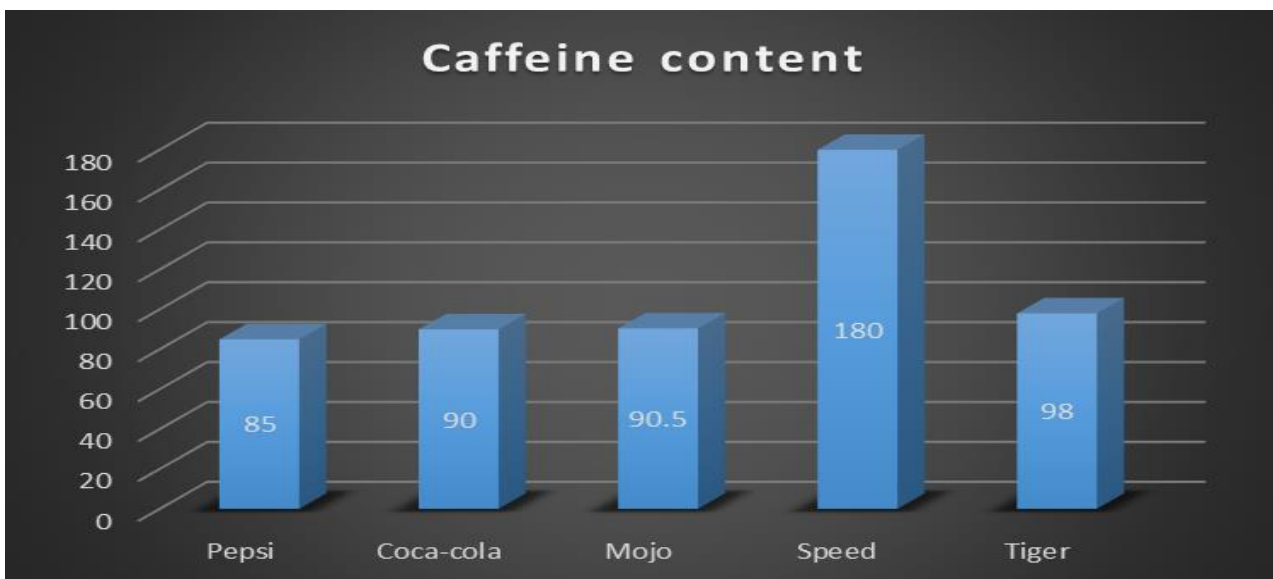
. The minimum caffeine content was found in sample 3(85mg per 250ml drinks),where the maximum amount of caffeine was found in sample 2 (180mg per 250ml drinks). The concentration of caffeine in all energy drink samples are well below the maximum allowable limits set by the food regulatory bodies, except the sample 2

5.2.7:   Caffeine Vs pH :

Brand name	Serving size	Caffeine content mg/l	pH
Coca-cola	250ml	90mg	2.45
speed	250ml	180mg	2.30
pepsi	250ml	85mg	2.43
Tiger	250ml	98mg	2.35
Mojo	250	90.5mg	3

Caffeine is the most common thing for the soft drinks because most of the soft drinks contain caffeine except the clear or non-color soft drinks . if any soft drinks contains higher level of caffeine content ,then it is very harmful for our body. A normal person can consume 250-300mg/l caffeine in a day .from this study ,able to find that 2 of the samples contain higher caffeine content with lower pH and other three sample contain average amount of caffeine which is equal or less than standard limit

5.2.8:Sample caffeine content vs Standard caffeine limit:

Brand name	Serving size	Caffeine content mg/l	Standard limit
Coca-cola	250ml	90mg	90mg
speed	250ml	180mg	90mg
pepsi	250ml	85mg	90mg
Tiger	250ml	98mg	90mg
Mojo	250	90.5mg	90mg
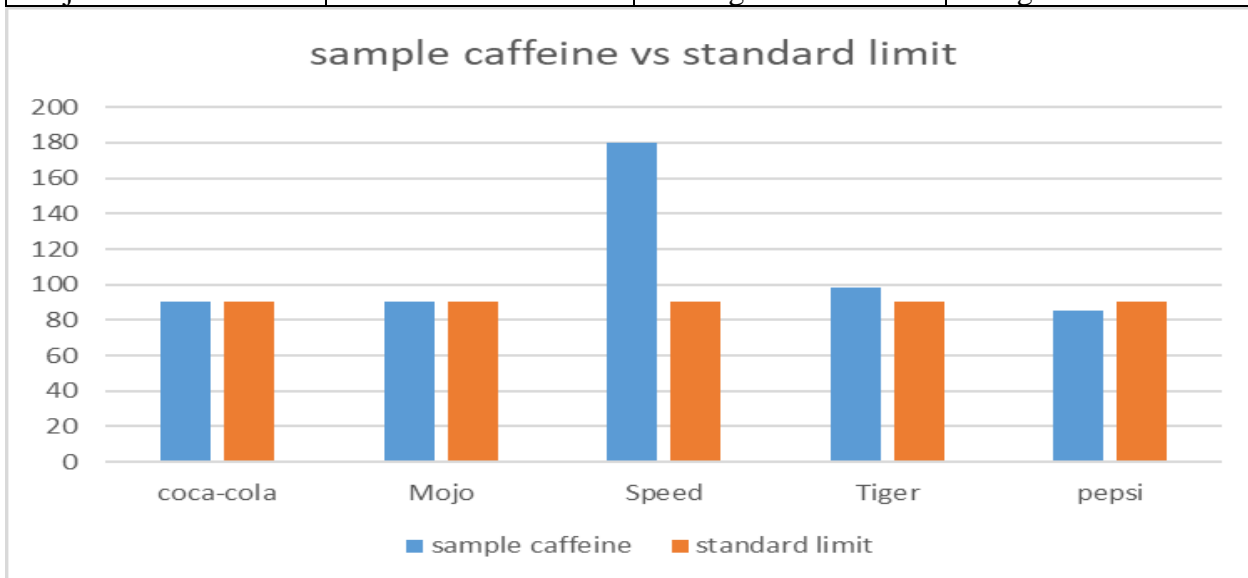
As observed that almost al the sample contain the caffeine within the standard rage or slightly upper to the standard range except the Sample S which contained

5.2.9:Observed Lead value in all samples :

Sample No	Sample Name	Abs	Conc	Actual
1	Blank	0.0158	2.0094	
2	Sample T	0.0133	1.6632	0.3462
3	Sample CC	0.0276	3.6435	1.6341
4	Sample S	0.0210	2.7295	0.7201
5	Sample M	0.0135	1.6909	0.3185
6	Sample P	0.0966	13.1985	11.1891

As observed that all the contained lead metals which are mainly contaminated by the water .all the samples were in WHO limit except sample P which contained 11.1891mg/l which is much higher than the WHO limit .

5.2.10: Observed Cadmium value in all samples :

Sample No	Sample Name	Abs	Conc	Actual
1	Blank	0.0036	0.0339	
2	Sample T	0.0061	0.0452	0.0113
3	Sample CC	0.0058	0.0439	0.0100
4	Sample S	0.0047	0.0389	0.0050
5	Sample M	0.055	0.0425	0.0086

| 6 | Sample P | 0.0050 | 0.0402 | 0.0063 |

Cadmium was observed in all the samples .and all the samples contained Cadmium range from 0.063 to 0.0113 mg/l which are in limit of WHO .So there is no significant difference among the samples .
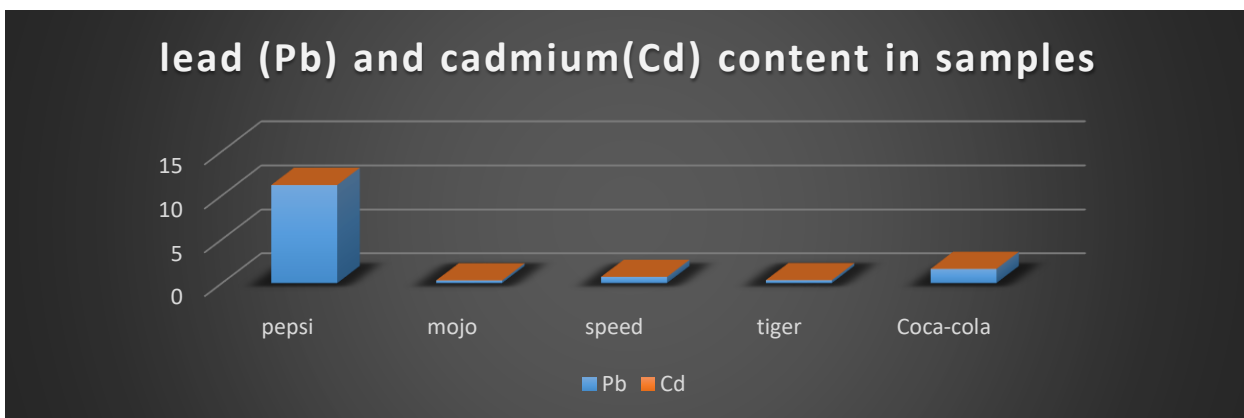

Figure : Pb and Cd content in all samples

Water is the main element for the any kind of drinks or juice .if the water is not purified properly during the production process ,soft drinks may be contaminated by the heavy metals like lead,cadmium ,nickel ,chromium ,cobalt etc . in this study cadmium and lead were present in the all samples quantities varies significantly among various sample.

CHAPTER SIX

CONCLUSIONS

Conclusion

As it was an attempt of academic research ,it had not done at larger scale .The data presented in this gave a preliminary outline about the content levels in soft and energy drinks beverage's frequently consumes in Bangladesh .Based on the data ,the level of caffeine content are within or higher than the maximum authorized levels .almost every samples contain higher caffeine contents with lower pH value that is the main concern,

Almost every sample were acidic in nature due to the their lower pH , soft drinks which are high in caffeine content ,low in pH is very harmful for human body because caffeine is alkaloid which is more active in the acidic condition for this reason it weakens human's central nervous system .according to the FDA limit ,300mg caffeine per day is safe human's health, especially for the pregnant women and children . Study showed that almost every soft drinks samples contain higher amount of caffeine content ,so can say that mentioned sample in the study should not consume daily .

Study showed that all the samples contain the lead and cadmium. Most of the samples contain lead and cadmium within the range except few samples .Cadmium level was in the authority limit in all the samples .whereas Lead content also in the standard limit in most of the samples except Sample P which showed 11mg/l which is more higher than the food regulatory bodies limit, that is not consistent with limit specified by the appropriate bodies. This amount is totally abnormal, this abnormalities may arise from the interference it may be from the chemical interference or background interference .were not able to repeat the test due to the lack of time and availability of AAS instrument and also financial issue as well .

As the study had done within small range ,so further investigation is required to ensure that all samples of the soft and energy drinks follow the limit of food regulatory bodies .

CHAPTER SEVEN

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