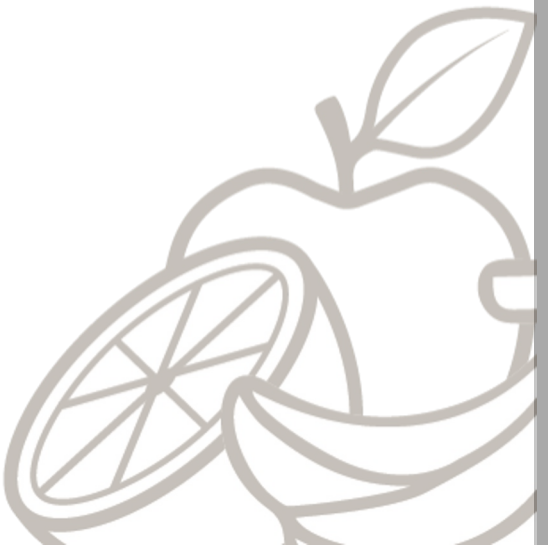
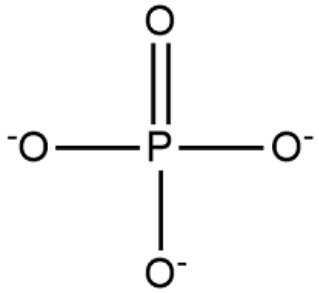
BCH 445- Biochemistry of Nutrition [Practical]

Estimation of inorganic phosphate in soft drinks



Phosphate in food

- Phosphate occurs **naturally** in the form of **organic esters** in many kinds of food, including meat, potatoes, bread, and milk.
- Phosphate also used as a food additive (inorganic phosphate) as a preservative, a flavor or color enhancer, extend shelf life, and retain moisture.



Phosphate

Figure 1. Chemical structure of phosphate

Soft drinks

- Soft drinks are complex mixtures containing a variety of substances such as coloring compounds, flavoring agents, acidifiers, sweeteners, preservatives, and caffeine.
- The most common **acidifier used in soft drinks** is phosphoric which gives a tangy taste in the mouth.
- Phosphoric acid can also acts as a preservative, keeping the contents of the bottle fresh.

Brand	Ingredients (as listed by the manufacturers)	Volume (mL)
A	Carbonated purified water, high-fructose syrup, sucrose, cane sugar, phosphoric acid, nature flavorings, caffeine	600

Figure 2. Ingredients of soft drinks

Coke vs Vinegar

Due to the use of phosphoric acid, cola has similar acidity to vinegar which no body can drink straight, but a ton of sugar, dyes and flavoring are added to **mask the acidity**.

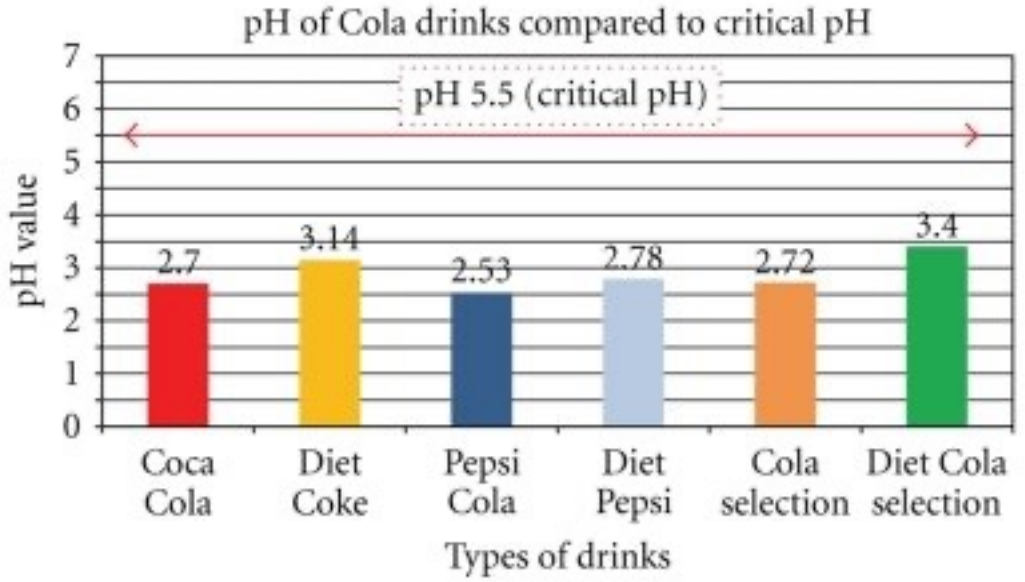


Figure 3. pH comparison among pop-Cola drinks

Practical Part

Objective:

- Estimation of inorganic phosphate in soft drinks using ascorbic acid as a reducing agent.

Ammonium molybdate solution is prepared in sulphuric acid, safety goggles and gloves should be worn when handling the reagent

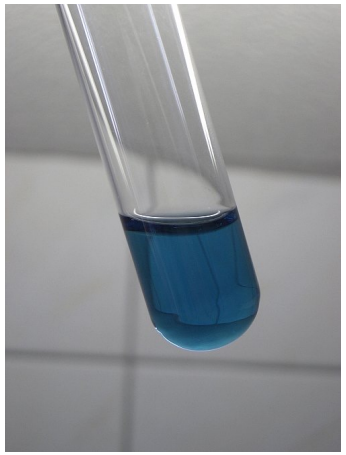
Principle

- Phosphoric acid is colorless, it **cannot be directly** determined using visible-light spectrophotometry, instead we'll quantitatively convert them into a colored substance, whose absorbance can be easily measured.
- Inorganic phosphate reacts with ammonium molybdate in an acid solution (ammonium molybdate prepared in sulphuric acid in this experiment) to form phosphomolybdic acid.
- Phosphomolybdic acid is then reduced by a reducing agent (3% ascorbic acid) to give **molybdenum blue a green/ blue color** that absorbs at 650nm .

Phosphate + Ammonium molybdate $\xrightarrow{H^+}$ Phosphomolybdic acid

Phosphomolybdic acid + 3% Ascorbic acid $\longrightarrow$ **molybdenum**

(λmax= 650nm)



Method

	Standard	Soft drink sample	Water	Ammonium molybdate	Ascorbic acid
Blank	------	------	2	0.5 ml	0.5 ml
3 ppm	2	------	------		
4.5 ppm	2	------	------		
6 ppm	2	------	------		
12 ppm	2	------	------		
15 ppm	2	------	------		
Sample (try different concentration)	-------	2	0		
		1	1		

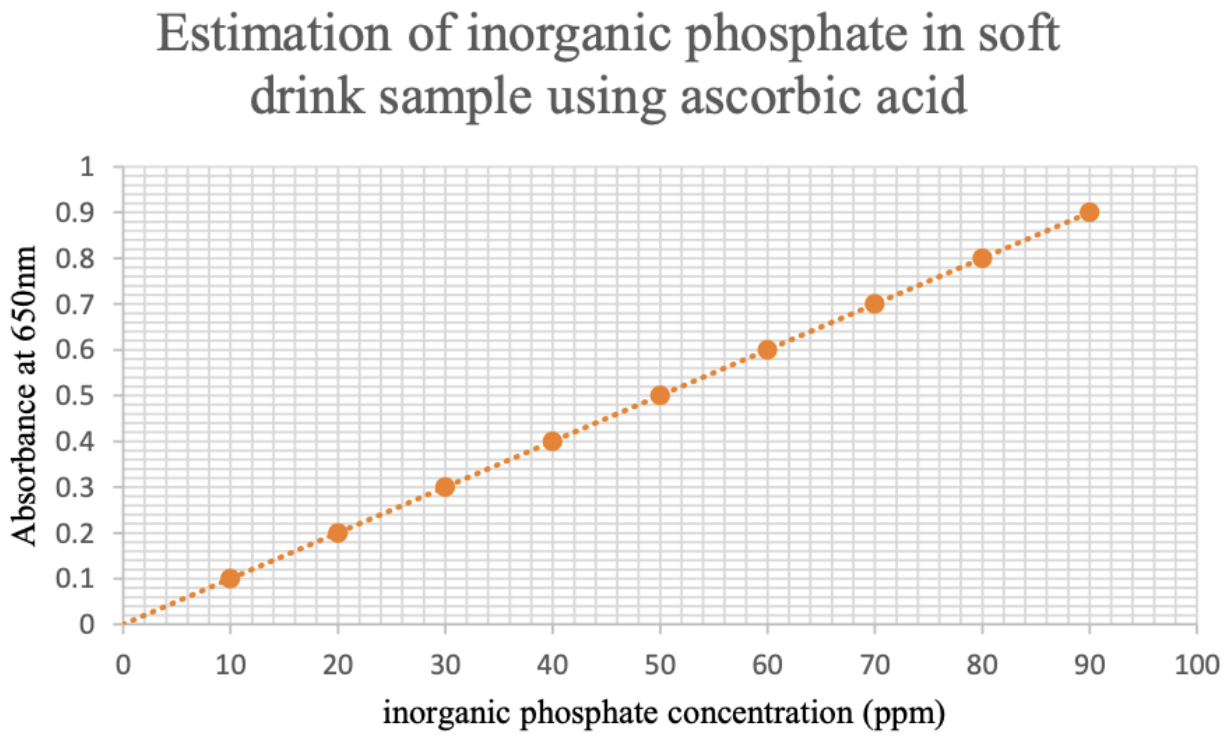
Mix **thoroughly** after each addition → Allow to stand for 10 min

(a deep blue/green color should develop) → Measure the absorbance at 650 nm.

Results

- Plot a graph between absorbance and concentration of phosphate in various standard solutions and obtain the calibrated curve.
- From the curve determine the amount of phosphate in the test solution.

Tube	Absorbance at 650 nm
Blank	
3 ppm	
4.5 ppm	
6 ppm	
12 ppm	
15 ppm	
Sample	



Calculations

- **Inorganic phosphate concentration=** dilution factor x concentration from the curve = ------- ppm
- **Dilution factor=** final volume / aliquot volume

SD1=

SD2=

Note: the original sample was diluted 1:50

Homework

1. Enumerate 3 health effects of soft drinks.
2. How soft drinks consumption lead to osteoporosis?