Determination of Vitamin C Content in Vitamin Beverages and Vitamin C Effervescent Tablets by 2,6-Dichloroindophenol Method

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Abstract

This paper is a biochemistry design experiment thesis from Harbin Institute of Technology (Weihai). The experiment employed the 2,6-dichloroindophenol method to determine the vitamin C content in vitamin beverages and effervescent tablets, discussed the feasibility of using pre-made vitamin beverages and effervescent tablet solutions as vitamin C supplements, and aimed to provide a reference for related biochemistry course design experiments. The results indicated that Mizone beverage contains 0.566 mg of reduced vitamin C per milliliter, while effervescent tablets contain 84.86 mg of reduced vitamin C per gram. From the perspective of vitamin C supplementation, daily consumption of a 1 L bottle of Mizone beverage or one effervescent tablet does not exceed the maximum intake limit for vitamin C, making them excellent choices for vitamin C supplementation beyond dietary sources.

Full Text

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Abstract

This paper presents a biochemistry design experiment conducted at Harbin Institute of Technology (Weihai), employing the 2,6-dichlorophenol indophenol method to determine vitamin C content in vitamin beverages and effervescent tablets. The study evaluates the feasibility of using ready-to-drink vitamin beverages and effervescent tablet solutions as vitamin C supplements, aiming

to provide a reference for similar biochemistry course design experiments. Results indicate that Mizone beverage contains 0.566 mg of reduced vitamin C per milliliter, while vitamin C effervescent tablets contain 84.86 mg per gram. From a supplementation perspective, consuming one 1-liter bottle of Mizone beverage or one effervescent tablet daily does not exceed the upper intake limit for vitamin C, making them excellent choices for dietary supplementation, though total intake from all sources should be monitored.

Keywords: 2,6-dichlorophenol indophenol method; Vitamin C; Vitamin beverages; Vitamin C effervescent tablets

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Introduction

Vitamin C, also known as L-ascorbic acid (Vc), is a water-soluble vitamin and one of the most critical nutrients for human health. Deficiency leads to scurvy, earning it the name antiscorbutic acid, and it also plays a vital role in immune modulation [**?**]. As a powerful reducing agent, vitamin C is readily oxidized to dehydroascorbic acid, though this reaction is reversible and both forms maintain physiological activity. However, further oxidation of dehydroascorbic acid to diketogulonic acid is irreversible and results in complete loss of biological function [**?**].

In recent years, an increasing number of beverages have been fortified with vitamin C to enhance their nutritional value, making such drinks a significant source of vitamin C supplementation for many consumers. However, more vitamin C is not necessarily better. Short-term excessive intake can cause diarrhea or skin rashes, while chronic overconsumption may lead to decreased immunity and kidney stones. The Chinese Nutrition Society recommends a daily intake of 100 mg for adults, with an upper limit of 1000 mg per day.

Consequently, determining the vitamin C content in various food products, particularly fortified beverages, is both important and necessary. Numerous methods exist for this purpose, including the 2,6-dichlorophenol indophenol method [**?**], iodometric titration [**?**], high-performance liquid chromatography [**?**], atomic absorption spectroscopy [**?**], spectrophotometry [**?**], capillary electrophoresis [**?**], and fluorescence quenching [**?**]. This study selected the 2,6-dichlorophenol indophenol method for determining vitamin C content in both ready-to-drink beverages and solid effervescent tablets. The principle relies on the color change

of 2,6-dichlorophenol indophenol dye, which appears blue in alkaline solution and red in acidic solution, becoming colorless upon reduction. When titrating an acidic vitamin C solution, each drop of dye is immediately reduced to its colorless form until all vitamin C is oxidized. The endpoint is reached when a faint pink color persists for 15 seconds, allowing calculation of reduced vitamin C content based on dye consumption. This method offers simplicity, rapidity, and accuracy without requiring specialized instrumentation.

This investigation examined commercially available peach-flavored Mizone vitamin beverage and vitamin C effervescent tablets to quantify their vitamin C content and assess the feasibility of these products as vitamin C supplements, providing scientific guidance for rational nutrient supplementation and dietary planning.

2. Materials and Methods

2.1 Experimental Materials

- Commercially available Mizone vitamin beverage, 1 L bottle (peach flavor)
- Commercially available vitamin C effervescent tablets (solid beverage containing effervescent disintegrants, OEM branded)

2.2 Reagents and Equipment

1. 1% oxalic acid solution
2. 2% oxalic acid solution
3. Standard ascorbic acid solution (0.1 mg/mL)
4. 0.05% 2,6-dichlorophenol indophenol solution
5. Electronic balance
6. Alkaline burette
7. Volumetric flasks (100 mL, 1000 mL)
8. Pipettes
9. Erlenmeyer flasks

2.3 Experimental Methods and Procedures

2.3.1 Standardization of 2,6-Dichlorophenol Indophenol Solution Using a pipette, accurately transfer 4.00 mL of standard ascorbic acid solution into a 100 mL Erlenmeyer flask, then add 16.0 mL of 1% oxalic acid solution. Fill an alkaline burette with the standardized 2,6-dichlorophenol indophenol solution and record the initial reading. Titrate dropwise until the solution turns faint pink and maintains this color for 15 seconds, indicating the endpoint. Record the volume of dye solution consumed and repeat the procedure three times to calculate the mass of ascorbic acid oxidized per milliliter of dye solution.

2.3.2 Determination of Vitamin C Content in Mizone Beverage Measure 10.0 mL of Mizone beverage and 90.0 mL of deionized water into a 250 mL

beaker. Using a pipette, accurately transfer 10.00 mL of the diluted sample to an Erlenmeyer flask. Fill the alkaline burette with standardized 2,6-dichlorophenol indophenol solution, record the initial reading, and titrate dropwise until a faint pink color persists for 15 seconds. Record the volume of dye solution used and repeat the titration three times to calculate the vitamin C content in the beverage.

2.3.3 Determination of Vitamin C Content in Effervescent Tablets Weigh one vitamin C effervescent tablet on an analytical balance, then dissolve it completely in 100 mL of deionized water in a 250 mL beaker. Pipette 1.00 mL of this solution into an Erlenmeyer flask, add 19.0 mL of deionized water, and titrate with standardized 2,6-dichlorophenol indophenol solution until a faint pink color persists for 15 seconds. Record the volume consumed and repeat the procedure three times to calculate the vitamin C content per tablet.

2.3.4 Blank Titration Pipette 20 mL of 1% oxalic acid solution into a 100 mL Erlenmeyer flask and titrate with 2,6-dichlorophenol indophenol solution until a faint pink color appears. Perform this blank titration in triplicate for control purposes.

3. Results

3.1 Blank Control

Table 1: Blank titration data (initial reading mL, final reading mL, difference mL)

3.2 Standardization Results

Table 2: Standardization of 2,6-dichlorophenol indophenol solution (initial reading mL, final reading mL, difference mL)

3.3 Vitamin C Determination Results

Table 3: Vitamin C content determination in Mizone beverage (initial reading mL, final reading mL, difference mL)

Table 4: Vitamin C content determination in effervescent tablets (initial reading mL, final reading mL, difference mL)

The results demonstrate that Mizone beverage contains 0.566 mg of reduced vitamin C per milliliter, while effervescent tablets contain 84.86 mg per gram.

4. Discussion

The experimental results align with expectations and correspond to the vitamin C content declared on product labels. From a supplementation standpoint, consuming one 1-liter bottle of Mizone beverage or one effervescent tablet daily

does not exceed the upper intake limit for vitamin C, making them excellent options for dietary supplementation. However, attention must be paid to total vitamin C intake from all sources when using different supplementation forms.

5. Declaration and Acknowledgments

This paper represents a biochemistry design experiment from Harbin Institute of Technology (Weihai). The experimental methodology is relatively simple and data are limited, serving only as a reference for biochemistry laboratory course design and related research. We sincerely thank the teachers, classmates, and graduate students from the School of Marine Science and Technology at Harbin Institute of Technology (Weihai), particularly Ms. Xie Qiuju from our experimental center, for their meticulous guidance throughout the experimental design and implementation.

6. References

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Note: Figure translations are in progress. See original paper for figures.