Title: Is It Good Enough?

Key Question: Your physician has recommended that you take a vitamin B-6 supplement. You are comparing brands and prices and have noticed that there is wide variety of brands, quality and prices. Is there a difference? Which one should you get?

Science Subjects: biology, chemistry, Integrated Sciences

Grade and Ability level: 9-12

Science Concepts: Students will learn how different products containing common ingredients may or may not differ. Students will be able to understand the value of different products and product purity.

Overall Time Estimate: one to three days depending on teacher/student responsibilities.

Learning Styles: visual, kinesthetic, auditory

Vocabulary:

- Co-enzyme: a molecule that provides the transfer site for biochemical reactions required by an enzyme.
- Mass spectrometer: an instrument which can measure the masses and relative concentrations of atoms and molecules.
- Serial dilution: step wise dilution of substances in a solution
- Supplements: added to ones diet to compensate for a deficiency.

Lesson Summary: Students will be presented with the key question scenario and asked to make a prediction. This prediction will become their observation for an experimental design template. The template should resemble:

I. Observation: Is there a difference in the molecular integrity of Vitamin B6 from different sources?
II. Hypothesis: either a yes or no in terms of predicting the molecular integrity
III. IV: different brands  DV: molecular integrity
IV. Procedure: control will be pharmaceutical grade Vitamin B6
V. Results: per readings on mass spectrometer
VI. Conclusion: support or not of hypothesis and results
VII. Extensions: subjecting the molecule to different situations that might breach the integrity of the molecule.

Student Learning Objectives with Standards:

SC.912.L.18.11: Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity. Cognitive Complexity: Level 2: Basic application of Skills and Concepts.


Materials:

1. Portable Mass Spectrometer with accompanying equipment and materials
2. Pharmaceutical grade Vitamin B6
3. Variety of store brand Vitamin B6 products
4. Mortar and pestle
5. Micro-pipettes
6. Test tubes

Background Information:

Name: Vitamin B6, pyridoxine C8H11NO3 Molecular weight: 169 g/l

Role in metabolism: co-enzyme used for over 100 enzyme reactions mostly concerned with protein metabolism. Also plays a role in cognitive development through biosynthesis of neurotransmitters and in maintaining normal levels of the amino acid homocysteine. Also
involved with gluconeogenesis and glycogenolysis, immune function (promotions of lymphocytes and interleukin-2)

Recommended Intakes

Table 1 lists the current RDAs for vitamin B6 [1]. For infants from birth to 12 months, the FNB established an AI for vitamin B6 that is equivalent to the mean intake of vitamin B6 in healthy, breastfed infants.

Table 1: Recommended Dietary Allowances (RDAs) for Vitamin B6

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
<th>Pregnancy</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to 6 months</td>
<td>0.1 mg*</td>
<td>0.1 mg*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–12 months</td>
<td>0.3 mg*</td>
<td>0.3 mg*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–3 years</td>
<td>0.5 mg</td>
<td>0.5 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–8 years</td>
<td>0.6 mg</td>
<td>0.6 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9–13 years</td>
<td>1.0 mg</td>
<td>1.0 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–18 years</td>
<td>1.3 mg</td>
<td>1.2 mg</td>
<td>1.9 mg</td>
<td>2.0 mg</td>
</tr>
<tr>
<td>19–50 years</td>
<td>1.3 mg</td>
<td>1.3 mg</td>
<td>1.9 mg</td>
<td>2.0 mg</td>
</tr>
<tr>
<td>51+ years</td>
<td>1.7 mg</td>
<td>1.5 mg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Adequate Intake (AI)

Sources of Vitamin B6

Food

Vitamin B6 is found in a wide variety of foods. The richest sources of vitamin B6 include fish, beef liver and other organ meats, potatoes and other starchy vegetables, and fruit (other than citrus). In the United States, adults obtain most of their dietary vitamin B6 from fortified cereals, beef, poultry, starchy vegetables, and some non-citrus fruits. About 75% of vitamin B6 from a mixed diet is bioavailable.

Dietary supplements

Vitamin B6 is available in multivitamins, in supplements containing other B complex vitamins, and as a stand-alone supplement. The most common
vitamin B6 vitamin in supplements is pyridoxine (in the form of pyridoxine hydrochloride [HCl]). Absorption of vitamin B6 from supplements is similar to that from food sources and does not differ substantially among the various forms of supplements. Although the body absorbs large pharmacological doses of vitamin B6 well, it quickly eliminates most of the vitamin in the urine.

About 28%–36% of the general population uses supplements containing vitamin B6. Adults aged 51 years or older and children younger than 9 are more likely than members of other age groups to take supplements containing vitamin B6.

Vitamin B6 Deficiency

Isolated vitamin B6 deficiency is uncommon; inadequate vitamin B6 status is usually associated with low concentrations of other B-complex vitamins, such as vitamin B12 and folic acid. Vitamin B6 deficiency causes biochemical changes that become more obvious as the deficiency progresses.

Vitamin B6 deficiency is associated with microcytic anemia, electroencephalographic abnormalities, dermatitis with cheilosis (scaling on the lips and cracks at the corners of the mouth) and glossitis (swollen tongue), depression and confusion, and weakened immune function.

Individuals with borderline vitamin B6 concentrations or mild deficiency might have no deficiency signs or symptoms for months or even years. In infants, vitamin B6 deficiency causes irritability, abnormally acute hearing, and convulsive seizures.

End-stage renal diseases, chronic renal insufficiency, and other kidney diseases can cause vitamin B6 deficiency. In addition, vitamin B6 deficiency can result from malabsorption syndromes, such as celiac disease, Crohn's disease, and ulcerative colitis. Certain genetic diseases, such as homocystinuria, can also cause vitamin B6 deficiency. Some medications, such as antiepileptic drugs, can lead to deficiency over time.
Vitamin B6 and Health

Different clinical trials for health benefits of adding B6 as a supplement have mixed results. Trials regarding cardiovascular health benefits have included B6 with other supplements. Trials for improving cognitive functioning and cancer prevention also have mixed results. There are other studies regarding the role of B6 for premenstrual syndrome and nausea and vomiting in pregnancy.

Advanced Preparation:

1. Set up of mass spectrometer and acquisition of accompanying materials per teachers manual
2. Create a serial dilution of vitamin B6
   a. Molecular weight is 169 g/mol
   b. 1 molar solution = 169 grams of B6 with distilled water to make 1 liter.
   c. This can be obtained by using the following method;
      i. Crush enough vitamin B6 tablets to obtain 100 ml of B6 and dissolve in 100 ml of distilled water. This will give a solution in the range of $10^{-3}$
      ii. $\frac{1g}{1M} / \frac{169g}{1l} = 0.0059\text{ M}$

   Dilute this further to $10^{-6}$

3. Depending on the skill level of the students, a serial dilution of the supernatant of the vitamin needs to be made
   a. Dilutions are made by taking 10 ml of the supernatant and placing in a test tube. Add 90 ml of ddH$_2$O, this will be $10^{-4}$. Do this 2 more times to obtain $10^{-6}$ magnitude.
4. Prepare your $10^{-6}$ sample for the mass spectrometer per teacher instructions.
5. Students will create a table with results and share their results with the class. Conclusion will be based upon results.
6. Extensions will be created by choosing the determining if improper storage or the expiration date effects the integrity of the molecule

Procedure with Discussion Questions and Time Estimates:

Day one: One classroom period of 47 minutes. Allowing students to create an experimental design regarding the key question. Includes a class discussion of what vitamin B6 is, the role as an enzyme and the molecular structure.

Day two: One classroom period of 47 minutes. Students create a supernatant of the vitamin and a serial dilution to $10^{-6}$

Day three: On classroom period: students run samples, record results, make conclusions, create extensions using their stock solution

Assessment Suggestions:

1. Grading rubric on Experimental Design
   a. Correct ExD template followed: 10 pts.
   b. Proper labelling of control, IV and DV 10 pts.
   c. Following correct procedure for mass spec 10 pts.
   d. Posting results 10 pts.
   e. Creating new ExD with extension 10 pts.
      50 pts.

Extensions: Students should be able to generate other uses for the mass spectrometer in lines of measuring the integrity of the molecular structure of known substances.

- Looking for pollutants and contaminants
- Comparing other pharmaceuticals
- Identifying potential contaminants and pollutants in the food industry

Resources/references:

http://ods.od.nih.gov/factsheets/VitaminB6-HealthProfessional/