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PRIMARY AND SECONDARY STANDARDS

29.1 INTRODUCTION

Often in our life we come across the word 'standard'. Whether it is the standard of bus service or behaviour or education, we use this term very commonly in our life. So why are we obsessed with standard? Is standard necessary in all spheres of our life? If the answer is yes then should we not follow a standard in our laboratory too? If yes, then next question arises how to prepare a standard in our lab.

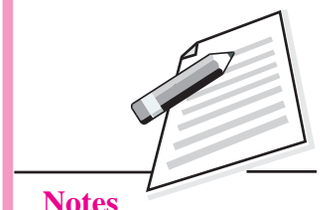
OBJECTIVES

After reading this lesson, you will be able to:

- define standard
- explain its uses
- classify standard with proper example
- explain various properties of standards

29.2 STANDARDS

In Biochemistry, the word standard means a material containing a substance of our interest with a known concentration. We can express this with definite numbers with proper units. By using this standard we can find out the concentration of that substance in a new material. Therefore, primary standard serves the purpose of being the primary calibrator or primary reference material.



1. Functions

Therefore, standard has the following uses in Biochemistry lab

(a) To provide a reference using which we can determine unknown concentration

(b) To standardize volumetric solutions

(c) Preparation of secondary standard

(d) To calibrate an instrument.

2. Types

Standards can be divided into two types:

1. Primary standard
2. Secondary standard

29.2.1. Primary standard

From the name itself it is obvious that this is a standard which comes first. That's why the name is primary.

A primary standard is a chemical or reagent which has certain properties such as

(a) It is extremely pure,

(b) Highly stable

(c) It is anhydrous

(d) It is less hygroscopic

(e) Has very high molecular weight

(f) Can be weighed easily

(g) Should be ready to use and available

(h) Should be preferably non toxic

(i) Should not be expensive

Having said that lets us understand each point one by one.

- A primary standard material should be extremely pure which means that it should be a chemical of high grade of purity, preferably 99.98%. In a chemistry lab you will come across chemicals of different grade of purity. If you check the label you will notice a number with percentage termed as purity. So when a chemical has purity of 99.98% or more it is a suitable material to be considered for primary standard.

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Usually those chemicals that exceed the requirement of American chemical society (ACS) are extremely pure and can be used for making primary standard. It is an analytical reagent of extreme purity which is specially manufactured for the purpose of being used as primary standard.

- It should be highly stable which means it usually does not react easily when kept in its pure form. Or in other words it should have very low reactivity. This is important because if a reagent reacts easily with atmospheric oxygen or water or changes its property over time then it is unreliable. We can never use such unstable and unreliable chemicals as standard.
- It should be anhydrous which means that it does not contain any water molecule in its molecular structure. For example, in a chemistry laboratory you will come across same chemical with different number of water molecules attached with it. Let us see the example of Epsom salt. The chemical name is magnesium sulphate. So we write the formula is MgSO4. But the chemical Epsom salt which is found in grocery or drug store is a chemical with formula MgSO4.7H2O. Therefore if you want to prepare a primary standard of magnesium sulphate you should purchase an anhydrous MgSO4 preferably an analytical reagent grade chemical and with purity greater than 99.98%. Remember such reagents are usually available with common vendors.
- Just being anhydrous is not sufficient. The chemical preferably should be less hygroscopic that is on opening the container it should not absorb water molecules from atmosphere. Why water should not enter into chemical? This will be clearer in the following point where it is explained how presence of water molecule can affect the simple calculation of standard concentration making the entire standardization procedure unreliable.
- Has very high molecular weight compared to its other similar forms. If we take the same example of Epsom salt you can understand this statement. Let us take 1 gram of $MgSO_4$ fit for making a primary standard and we call it salt A. Now take 1 gram of $MgSO_4.7H_2O$ fit for common uses and name it salt B. Now you compare the actual weight of magnesium sulphate to make a standard solution for both chemicals. The molecular weight of MgSO4 is 108 but for $MgSO_4.7H_2O$ it is 234.

  In case of 1 molecule of salt A the weight of actual $MgSO_4$ will be 108 atomic mass unit. But In case of 1 molecule of salt B the weight of actual MgSO4 will be 108 out of its total weight of 234 atomic mass unit.

  108 gram salt A ($MgSO_4$) will give 108 gram of $MgSO_4$

  So 1 gram $MgSO_4$ salt will give = 108/108 = 1 gram of $MgSO_4$

  But 234 gram salt B ($MgSO_4.7H_2O$) will give 108 gram of $MgSO_4$

  So 1 gram $MgSO_4.7H_2O$ salt will give = 108/234 = 0.461 gram of $MgSO_4$

Therefore if you by mistake make a standard out of salt B, you will actually be taking 0.461 gram of MgSO4 and calculating it as 1 gram. So with this faulty standard estimation of MgSO4 in other unknown solution will give less result than the actual concentration

Therefore it is important that primary standards must be anhydrous and of high molecular weight.

- It can be weighed easily because it is so pure that its weight is in fact a true representative of number of moles present in its actual weight.
- One of the uses of primary standard is to standardize a volumetric solution. That means they are used for standardization of titration of solutions. It can be used for titration of acids as well as bases. Let us see how a primary standard is used for titration.
  - In a biochemistry laboratory the most common standards for acid titration we use basic chemical such as sodium carbonate ($Na_2CO_{3)}$, Tris which is also known as trisaminomethane [$(CH_2OH)_3CNH_2$] etc. you should know that Tris is a very commonly used chemical in Biochemistry and Molecular biology lab.
  - For base titration we use potassium hydrogen phthalate [(KHP): $KHC_8H_4O_4$] etc.
  - Another type of titration is redox titration which is very common in biochemistry lab. For this we frequently use potassium dichromate ($K_2Cr_2O_7$) very often as primary standard. Sodium oxalate ($Na_2C_2O_4$) is also used for this purpose.
- The primary standard is used for calibration of secondary standard or for method validation against a definitive method and it corresponds to the true value of the substance analyzed.

29.2.2 Secondary standard

From the name itself it is obvious that this is a standard which comes second. That's why the name is secondary.

A secondary standard is used by standard laboratories such as companies involved in preparation of reagents, kits or laboratories responsible for producing quality control material for other labs. They use primary standard as the primary calibrator or primary reference material. Secondary standard in turn is used for the purpose of calibration of control material in smaller lab for analysis of unknown concentration of a substance. So basically, secondary standard serves the purpose of external quality control for smaller labs. This makes it essential that the secondary standard must first be standardized against the primary standard.

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- There are other points one has to remember. For preparing the secondary standard solution one must use aquous solution of high grade purity. It must be deionized if aquous solvent used is water. Without pure solvent, the standard solution prepared will be worthless (These points are also applicable for preparing primary standard solution).
- Similarly before using high grade chemicals one should also be vigilant and check for date of manufacture, expiry date, date of receipt of chemical, whether the conditions for its transport was followed or not, if the seal is not tampered with, its purity, standard reference material used etc.

  A secondary standard is a chemical or reagent which has certain properties such as

  (a) It has less purity than primary standard

  (b) Less stable and more reactive than primary standard

  (c) But its solution remains stable for a long time

  (d) Titrated against primary standard

  Usually a chemical fit for being a standard chemical yet does not meet the requirements of a primary standard
- The best example is anhydrous sodium hydroxide (NaOH). It is extremely hygroscopic. As soon as the bottle is opened NaOH starts absorbing moisture from atmosphere and within no time it becomes moist. You can experiment it in your lab. Take the NaOH bottle near an analytical balance. Place a Petridis and make its weight as zero (by using the tare button). Now open the container and place little NaOH crystal on it and quickly note the weight. Now keep the glass windows of the analytical balance open for few minutes and notice the gradual increase in its weight in terms of mg units. This is because the NaOH crystals absorb water molecule from air. (Remember to use a glass container such as a Petridis to weigh the chemical. Because, NaOH is a corrosive for metal panel of balance).
- Another example is potassium permanganate ($KMnO_4$) very often as secondary standard. It is a good oxidizing agent or in other words it is reactive so less stable. More often due to its reactivity, its own oxidized product manganese oxide (MnO_2) contaminates the content. That's why it is unsuitable for being a primary standard. But it can be used very well as a secondary standard.
- Next question is why is secondary standard called still a standard? This is so because secondary standard is used as a calibrator by smaller laboratories involved in actual analysis of unknown samples.

Primary and Secondary Standards

In our text we have used the term calibration. So what is calibration? This is the process by which we compare the measurements by a standard or an instrument (primary) with another standard or an instrument (secondary). By doing so, we try to eliminate any variation or difference in measurement by the secondary standard or an instrument. The other term for calibration is standardization. Have you noticed how the shopkeeper puts a standard weight on his balance to his right and adds potatoes or tomatoes etc to the left? In this case the weight is the standard and the vegetables are being standardized with equal measurement of for their weight. So if there is a mistake in the weight he uses (let us think it is less than what is written on it) on his right side we may be cheated and get less vegetable than we are paying for

- Calibration using titration: take the example of vitamin C estimation from lemon. For this the standard is ascorbic acid which is available in your laboratory. Take ascorbic acid and prepare a standard solution of known concentration (for example 40mg/dl). Next prepare a diluted solution of indicator called 2 Di -chloro indophenol (2- DCIP) and add ascorbic acid drop by drop till it is completely decolourized. Let us say we used 20 ml.

Fig. 29.1: Original colour of 2 DCIP indicator

100 ml ascorbic acid standard solution has 40 mg of ascorbic acid in it

1 ml of standard solution has = 40/100 = 0.4 mg ascorbic acid

Now 20 ml of standard solution has = 0.4 ×20 = 8 mg ascorbic acid

This says that our 2- DCIP requires 8 mg of pure ascorbic acid for its complete neutralization or titration.

Fig. 29.2: Lemon

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Next let us use our lemon. Weigh the lemon. Let us say it weighs 4 g. add lemon juice drop by drop. Let us say it took several drops of lemon juice for complete neutralization of 2- DCIP. Now weigh lemon and now it weighs 2 g. So it proves that 2g of lemon juice present in 4 g of lemon has 8 mg of ascorbic acid. Why 8 mg of ascorbic acid? Simple. This is so because; 8 mg of ascorbic acid is required to completely neutralization 2 DCIP. So from this we came to know that

4 g of lemon has 8 mg of ascorbic acid

So 100 g of lemon would contain = 50 mg of ascorbic acid

So concentration of ascorbic acid in our variety of lemon is 50 mg/100 g that is equal to 50 mg%.

- The secondary standard is used for calibration of control materials in a lab using a reference method and it is closer to the true value of the substance. So it is used for external quality control programme.
- Whereas in a clinical laboratory unknown samples are analyzed against control materials using a routine/ field method

Let us compare the situation in biochemistry laboratory dealing with human samples. Here, we often use the term primary and secondary calibrator instead of calling it primary and secondary standard. A primary calibrator is a preparation with a stated purity (> 99.98%). The reference measurement procedure is calibrated with primary calibrator. Using this reference measurement procedure the **Institute for Reference Materials and Measurements (IRMM)** determines the value of secondary reference materials.

Even the companies, which sell their kits and reagents to us, use these secondary reference materials. So their calibrator products are basically secondary reference materials. We use these secondary calibrators as reference in our laboratories. The primary calibrator is used only by institutes like IRMM which are involved in quality assurance at National level.

Preparing a primary standard out of a pure chemical is relatively easier (Remember? It is one of the criteria to be a primary standard.). For **biological analytes**, we can not prepare the secondary standard by dissolving the analyte in water. It is so because in our blood they are dissolved in plasma and not in water. For example, for plasma proteins, there is a **human reference serum material** which comes with a certificate of concentrations present for 12 serum proteins from IRMM.

Primary standard — **At the National level**

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Reference measurement procedure/Definitive method

↓ **At the Company level**

Secondary standard (prepared and supplied by company)

↓

Company measurement procedure/ Reference method

↓

Product calibrator = Secondary standard supplied by company (external quality control)

↓ **At the End-User level**

Routine procedure in lab/ Field method (standardization against Product calibrator)

↓

Value assignment/Internal quality control/analysis of sample etc.

Flow chart summarizing the use of primary and secondary standards in a clinical laboratory set up

INTEXT QUESTIONS 29.1

1. Standard can be of ..... types.
2. A primary standard should be ..... pure.
3. Between primary and secondary standards, ... standard is more reactive.
4. We can calibrate a ..... or ..... using primary standard.
5. Secondary standard can be prepared by ..... using primary standard.

WHAT HAVE YOU LEARNT

- A standard also called a calibrator is a material containing a substance of our interest with a known concentration, which can be expressed with definite numbers and units. It is of 2 types primary and secondary. Primary standard is purer, more stable, less reactive, anhydrous and less hygroscopic

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compared to secondary standard. Secondary standard is titrated or calibrated against primary standard and used as a reference material in all labs across country for the purpose of analysis. Preparation of standard solution should use pure, de-ionized aqueous solvent to keep the quality of standard intact. In a clinical lab set up, primary standard acts as a primary reference material. Secondary standard is used as an external quality control for calibration of internal quality control in labs. Unknown sample is analyzed after both external and internal quality check is complete and satisfactory.

TERMINAL QUESTIONS

1. What are the criteria of a primary standard?
2. What are the criteria of a secondary standard?
3. What are the differences between primary and secondary standards?
4. What are the uses of primary standard?
5. Why should the primary standard be of high molecular weight?
6. Why can't you use NaOH as a primary standard?
7. Why can't you use $KMnO_4$ as a primary standard?

ANSWERS TO INTEXT QUESTIONS

29.1

1. Two
2. >99.8%
3. Secondary
4. Secondary standard or an instrument
5. Titration