

Methodology Article

Color Presenting Products of Amino Acids Reactions- Qualitative Tests

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Abstract: This paper presents simple approach for systematic chemistry learning of amino acids' qualitative experiments. In this laboratory practice, students are requested to perform qualitative tests for certain amino acids and be able to distinguish them according to their color reactions. The chemical knowledge and concepts of amino acids reactions with certain reagents are deeply discussed to promote students' readiness for their future career as chemists and lab technicians. In this approach, all written laboratory activities are linked with colored reactions' illustrations to facilitate better understanding of all laboratory tests.

Keywords: Chemical Education, Color Reactions, Amino Acids, Qualitative Tests

1. Introduction

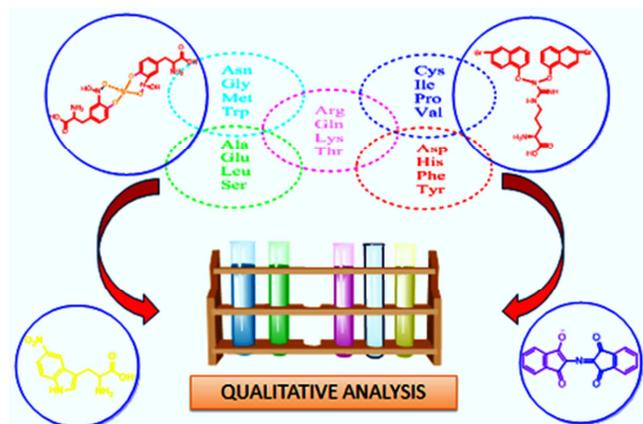


Figure 1. Graphical Abstract.

Amino acids are the building blocks of proteins, and are linked in series by peptide bonds to form the primary structure of proteins. They possess an amine group, a carboxylic acid group and a varying side chain that is unlike between different amino acids. There are 20 naturally occurring amino acids, which vary from one another by the structures of their side

chains. There are different approaches to highlight the implementation of laboratory work [1-6]. Most are meant for promoting undergraduate students to explore conceptual understanding of functional groups [7, 8]. Laboratory activities in the teaching of chemistry concepts to undergraduate students represent important component in the curricular development [9, 10]. These activities are implementing learning through interaction with materials and/or models for better understanding of the subject matter [11, 12]. These practices usually wake up students and encourage them to achieve a conceptual understanding of the required knowledge [8, 12].

Our main objective of this experimental work is to introduce the undergraduate students to the qualitative experiments of amino acids as a way of learning chemistry. The experiments are linked to the concepts of the reactions in order to promote the adoption of knowledge from a laboratory. These experiments are carried out in groups to stimulate and encourage collaboration and team work environment among students. Students are expected to link theory to practice and the role of the laboratory instructor here is to monitor the lab session and stimulates students to develop a cooperative work and to be able to communicate and explain their results.

2. Pedagogic Approach

Instead of having scattered information here and there, this article is meant to have simple, clear and accurate information related to color reactions of the qualitative tests of amino acids. In this lab practice undergraduate students can link theory with concepts through a simple knowledge promoting approach and smoothly and easily understand experiments that deal with one of the most important groups of biomolecules in organic chemistry called amino acids.

3. Methodology

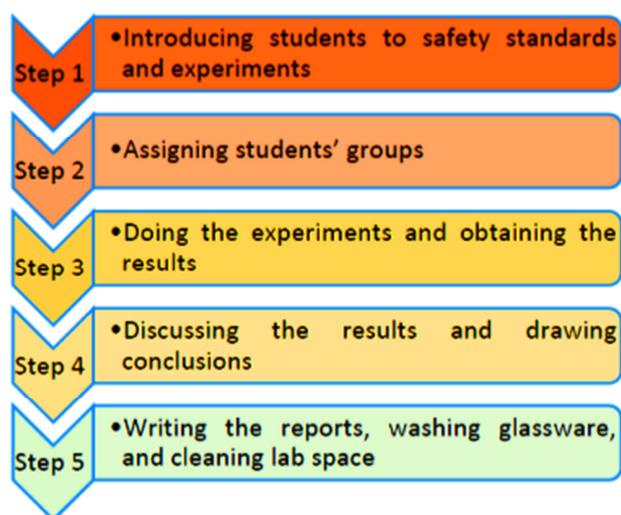


Figure 2. Methodological Laboratory Approach.

The methodological laboratory approach was divided into five steps shown below in Figure 2. The first step was

4. Laboratory Activities, Experimental Overview and Students' Proposed Tasks

Students do the following experiments: Ninhydrin Test, Xanthoproteic test, Millon's Test, Hopkins-Cole Test, Lead-Sulfide Test, and Sakaguchi test [13-15]. As described earlier [16], instructor provides a complete manual for the experiments as student's handout in line with the students' laboratory discussion. Experiments are designed and implemented as part of an industrial chemistry technology course, named as Introduction to Macromolecule Chemistry [13], for industrial chemistry undergraduates after the completion of organic chemistry (I) course as a prerequisite. Students are introduced to the theoretical concepts related to macromolecule chemistry chapter prior to the practical experiments. Students are also distributed into groups of 3 students each and the experiments are designed to be completed within three laboratory periods (≈ 2.5 h) as shown in table 1.

Table 1. Overview of the Students' Performance Tasks.

Task Description	Time (In Minutes)
a. Assigning students' groups	10
b. Distribution of handouts	05
c. Allocating experimental tasks	05
d. Labeling beakers, test tubes, preparation of reagents, solutions and performing selected experiments	90
e. Summarizing results, observations, and conclusions	20
f. Cleaning glassware and making place tidy	20
Total time for all tasks	150

5. Hazards

a. Ninhydrin is a strong oxidizing agent, it should be handled with care, and applied apart from contact with skin or eyes, gloves and mask is a must, using hood is required, if accidentally get in touch with the skin, the resulting stains is a temporarily one, that will be eliminated within 24 hours.

b. Concentrated nitric acid (HNO_3) is a toxic, corrosive substance that can cause severe burns and discolor your skin.

Prevent eye, skin and cloth contact. Avoid inhaling vapors and ingesting the compound. Gloves and safety glasses are a must; the test is to be performed in a fume hood.

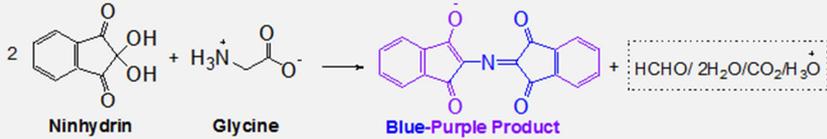
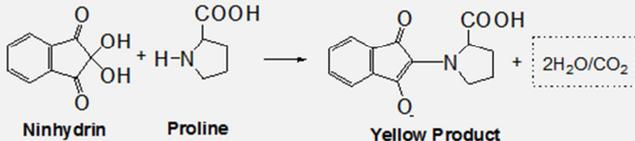
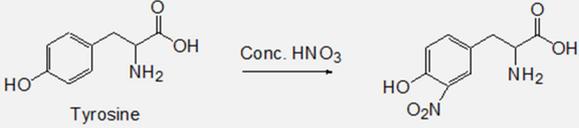
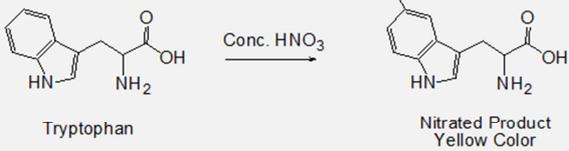
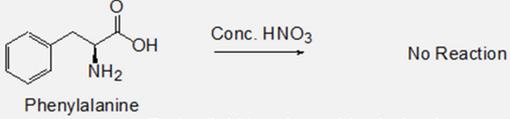
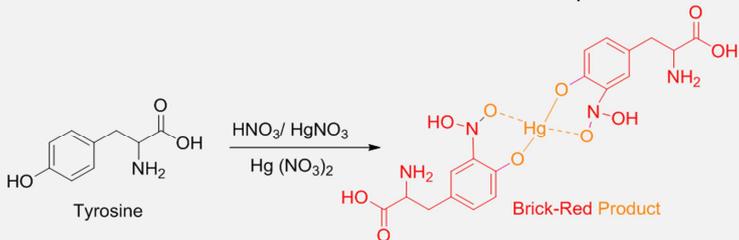
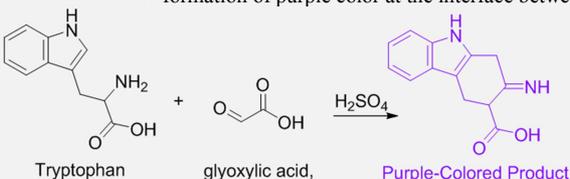
c. MILLON'S reagent is highly toxic and highly corrosive.

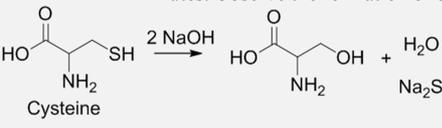
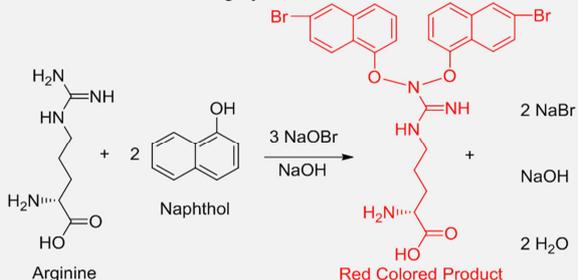
d. Sulfuric acid (H_2SO_4) causes severe skin burns and eye damage. Prevent eye, skin and cloth contact.

e. Sodium hydroxide itself is highly reactive and its solution is a colorless liquid. More dense than water. Contact may severely irritate skin, eye, and mucous membranes. Toxic by ingestion and corrosive to metals and tissues.

6. Experimental

Table 2. Ninhydrin, Xanthoproteic, Millon, Hopkins-Cole, Lead-Sulfide, and Sakaguchi tests.

Tests	Specific group of amino acid	Procedure	Product Color
Ninhydrin	For all α -L amino acids (primary amine) and proline (secondary amine)	To 0.5 mL 2% amino acid solution add 5-10 drops of 0.2% Ninhydrin solution in acetone or ethanol. Heat in water bath for 2-5 minutes. Allow to cool and observe the formation of the colored products.	Blue-Purple OR Yellow
		 <p>Ninhydrin + Glycine \rightarrow Blue-Purple Product + HCHO/2H₂O/CO₂/H₃O⁺</p>  <p>Ninhydrin + Proline \rightarrow Yellow Product + 2H₂O/CO₂</p>	
Xanthoproteic	Tyrosine and Tryptophan (aromatic ring)	To 1 mL 2% amino acid solution in a test tube, add equal volume of concentrated HNO ₃ . Heat over a flame for 2 minutes or in water-bath for 15-20 minutes and observe the color. Cool thoroughly under the tap and CAUTIOUSLY run in sufficient dropwise 20-40% NaOH to make the solution strongly alkaline. Observe the formation of the yellow colored product.	Yellow
		 <p>Tyrosine $\xrightarrow{\text{Conc. HNO}_3}$ Nitrated Product (Yellow Color)</p>  <p>Tryptophan $\xrightarrow{\text{Conc. HNO}_3}$ Nitrated Product (Yellow Color)</p>  <p>Phenylalanine $\xrightarrow{\text{Conc. HNO}_3}$ No Reaction</p>	
Millon	Tyrosine (phenolic group)	To 1 mL 2% amino acid solution in a test tube, add 1-3 drops of Millon's reagent. Warm the tube in a boiling water bath for 10 minutes. Observe the formation of the brick red colored product.	Brick-Red
		 <p>Tyrosine $\xrightarrow{\text{HNO}_3/\text{Hg(NO}_3)_2}$ Brick-Red Product</p> <p>Structure Proposed By Dr. Elzagheid</p>	
Hopkins-Cole	Tryptophan (indole ring)	To 1 mL of Hopkins-Cole reagent, add 1 mL of 2% amino acid solution. Pour 1-2 mL H ₂ SO ₄ down the side of the sloping test tube. Observe the formation of purple color at the interface between the two layers.	Purple
		 <p>Tryptophan + glyoxylic acid $\xrightarrow{\text{H}_2\text{SO}_4}$ Purple-Colored Product</p>	
Lead-Sulfide	Cysteine, cystine, and methionine (sulfhydryl/thiol group)	Add 5 mL 20% NaOH to 2 mL 20% lead acetate. A white precipitate of lead hydroxide forms. Boil until the precipitate dissolves with the formation of sodium plumbate. Boil 1 mL 2% amino acid solution with a few drops of 40% NaOH for 5-10 minutes. Cool and add a few drops of	Dark precipitate

Tests	Specific group of amino acid	Procedure	Product Color
		<p>the sodium plumbate solution. Observe the formation of dark lead sulfide precipitate.</p> <p>OR</p> <p>Mix 6 drops of 2% amino acid solution with 2 drops of 40% NaOH solution and 1-2 drops of 20% lead acetate solution. Heat tubes for 10-20 minutes. Observe the formation of dark lead sulfide precipitate.</p>	
		 <p style="text-align: center;">Cysteine</p> $\text{Pb}(\text{CH}_3\text{COO})_2 + 2 \text{NaOH} \longrightarrow \text{Pb}(\text{ONa})_2 + 2 \text{CH}_3\text{COOH}$ $\text{Na}_2\text{S} + \text{H}_2\text{O} + \text{Pb}(\text{ONa})_2 \longrightarrow \text{PbS}\downarrow + 4 \text{NaOH}$ <p style="text-align: center;">Black ppt</p>	
Sakaguchi	Arginine (guanidino group)	<p>Mix 1 ml of 40% NaOH with 3 mL of 0.2% test solution (arginine), and add 2 drops of 1% α-naphthol. Add 4-5 drops of bromine solution and mix thoroughly. Observe the formation of red color.</p>  <p style="text-align: center;">Arginine</p> <p style="text-align: center;">Red Colored Product</p>	Red

7. Conclusions

This current work, which is based on the color reactions of amino acids, helps enriching the teaching-learning process of macromolecule chemistry experiments. The methodology proposed in this lab practice helps students in different practical aspects. The discussion of the reactions' topics offers a better understanding of the basic concepts, which allows guiding students in the rediscovery of scientific content. These laboratory practices enable students to compile, analyze and communicate their results. By this experimental approach students will understand various identification tests of amino acids and acquire good skills to perform those experiments in real laboratories.

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