Detection of Lycopene in Tomato Puree

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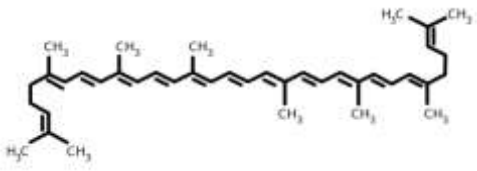
Abstract

Tomatoes are a very important part of daily diet around the world. Ripe tomatoes have an ingredient (a bright red caretonoid pigment) - lycopene - which has several beneficial health effects. To test the presence of lycopene in tomatoes is very easy using its light absorption characteristics. In this experiment a very simple technique is described which detects the presence of lycopene in tomato extract by detecting absorption of blue light.

Introduction



The main ingredient of pizzas is a sauce made from tomatoes. Tomatoes contain lycopene and β-carotene, which being antioxidants, are very good for health. A single lycopene molecule can neutralize as many as 13 free radicals which, if allowed to buildup, can cause cell damage and trigger cancer. This "busting power" of free radicals by lycopene is twice that of β-carotene. The amount of lycopene varies according to the variety of the tomatoes, but is highest in the red tomatoes. The red tomatoes can contain between 70 to 130 mg of lycopene per Kg of tomatoes depending on the variety, geographic location, technique of degree of ripeness of fruit. Lycopene and beta-carotenes break down on heating and are soluble in oil but not in water and, hence, in many parts of the world tomatoes are cooked in oil[1].

Fig.1 Structure of a Lycopene molecule

In order to test the presence of lycopene in tomatoes, tomato puree is dissolved in Petroleum-Ether and ethanol and the solution is allowed to settle. The lycopene-rich solution separates out, resulting in two immiscible liquids. The top layer is carefully separated out and its moisture content removed by using magnesium salts, which are hydroscopic in nature. Lycopene has a very unique characteristic in that it absorbs blue light (450-495 nm range) nearly completely.

Color	Approximate Wavelengths [nm]
Red	750 – 620
Orange	620 – 590
Yellow	590 – 570
Green	570 – 495
Blue	495 – 450
Indigo	450 – 420
Violet	450 – 380

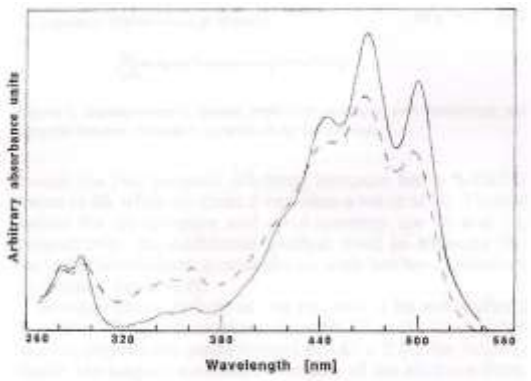


Figure 2. Absorption curves of lycopene in hexane: solid line, all-trans; dashed line, mixture of stereoisomers after isomerization by iodine and light.

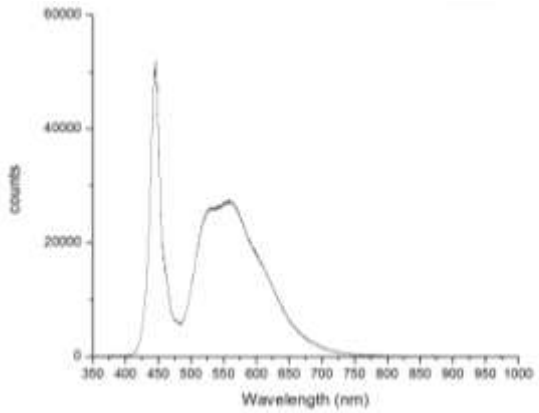


Figure 3. The spectrum of light emitted by a white LED, indicating the intensity of each of the wavelengths emitted by the white LED.

Figure 2 shows the absorption spectrum of Lycopene where it can be clearly seen that the absorption at 380 to around 500 nm is very high, which is the blue light region. Figure 3 shows the spectrum of light emitted by the white light LED used in this experiment. [This part of the

spectral information is not essential when the experiment is being carried out at high school levels. However, the information can be used by the teachers, as and when required.]

[In experiments, which involve several pieces of glass ware, it is easier for students if these pieces of hardware are given some names/labels. In IJSO, occasionally, glassware is labeled so that it becomes easier for students to work without getting confused with large amount of glassware and other smaller pieces of equipment. These names were the choice of the authors, readers can assign names convenient to their local needs.

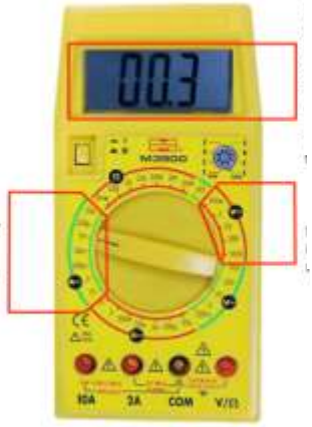
During the testing of the experiments for IJSO-2013, it was noticed by the authors, who were part of the academic committee designing these experiments, that students at this young age have difficulty using pipettes. Sucking chemicals/liquids in pipettes is not a healthy habit whereas using of rubber bulbs is not a very reliable option. So the committee decided to replace pipettes with syringes, and the experience of using them has been very successful. Thus in all experiments of IJSO, pipettes are now replaced by plastic syringes, readily and cheaply available in high-street medical supply stores.

The acrylic piece shown in the attached figure and used in this experiment has been described in details in a previous publication. Reference : IAPT bulletin, October, 2014, page 261. This equipment can be used in many other experiments which will be published in future.]

You are supplied with the following:

	Labelled as…	**Quantity Supplied**
Tomato Concentrate	**TP**	1 ml in 50 ml beaker
Extracting Solvent (1:1 Ethanol Petroleum Ether)	**ES**	(20ml) in 50 ml tube
Anhydrous Magnesium sulphate	**$MgSO_4$**	(1.5g) in a plastic container
Sodium chloride	**NaCl**	(15 gm) in plastic container
Test tube (50 ml) with stopper	**FL**	1
Test tubes (12 x 75 mm; 5 ml)	**Ab, UL**	2
Funnel		1
Glass rod		1
Filter paper		3
12 ml syringe	**SS**	1
Wash Bottle		1
White LED and		1

Photodiode acrylic set-up[2]		
50 ml beaker	**SS**	1
Test tube stand		1
Blue LED		1
Acrylic collar for test tubes	**Collar**	
Dropper		
Multimeter		

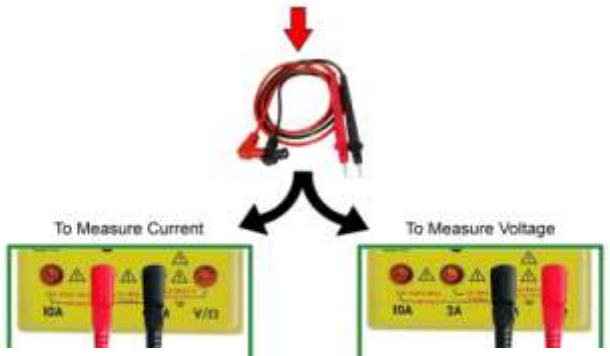


If display read *-1*, it implies range is insufficient. Shift the knob to higher range. Working in higher range than required will result in loss of accuracy

For this experiment DO NOT keep the knob in A ~V ~Ω

Multimeter knob is to be in this region for voltage measurement

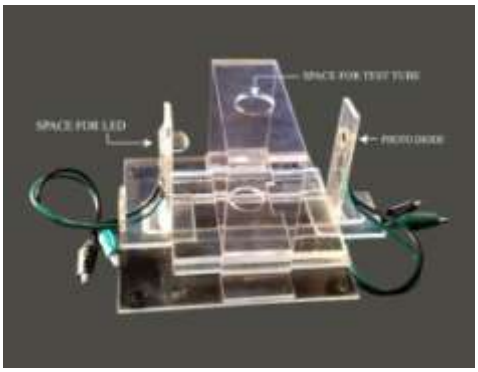
Multimeter knob is to be positioned in this region for current measurement
2µA is the minimum range of current measurement
10A is the maximum range



To measure current

To Measure Voltage

1. Press MODE button, if the Stopwatch is on Time Mode, to bring it to Stop Watch Mode. 0:00 00
2. Press SPLIT/RESET button to reset the Stopwatch to Zero.
3. Press START/STOP button to start the Stopwatch.
4. Press START/STOP button to stop the Stopwatch.
5. In Stopwatch mode START/STOP button should be used to start and stop as many times as required.



Stopwatch *Acrylic set-up with photodiode*

Procedure

1. Insert a white LED and photo-diode in their respective slots.

   [The absorption spectrum of lycopene and the spectrum of the white light LED may be discussed with the students for their additional information. However, the experiment can be carried out even without disseminating this information, if the teacher so chooses to do so]

2. Half-fill test tube **Ab** with solvent from tube **ES**, using a dropper.
3. Using the acrylic collar provided, place test tube **Ab** in the acrylic apparatus, such that it is located between the LED and the photodiode (*as shown in the photograph).*
4. Adjust the position of the photodiode and the test tube so as to maximize the current in the photodiode which is connected to the multimeter**.** Please ensure that the label on the test tube does not obstruct the light. This was discussed in the previous experiments of IJSO.

   [Here the teacher can observe the skill of the student. A careful student will ensure that issue raised in point 4 above is taken care of. Also a careful student will ensure that the LED light and the photo diode are facing each other, otherwise the light falling on the photodiode may not be maximum.

5. Measure the maximum current, I_s, and record your observation in **Table 1 in the answer sheet.**

   [It is not necessary that the maximum value of current is obtained when the LED and the photodiode are at the closest. This is because the light from the LED bends from the edges of the testtube to get focused at a point beyond the test tube. Thus the photodiode will record maximum current when it is placed at this point of "focus"]

   Note: Do not disturb the position of the photodiode and the acrylic test tube holder, it is crucial for subsequent readings.

   We shall now extract lycopene from tomato concentrate, as follows.

6. Stir the mixture in the beaker **TP** well with the glass rod and allow it to settle for 2-3 minutes. Wash the glass rod for further use.
7. Now, filter the solution carefully by using funnel, and filter paper, in test tube **FL**. The red clear solution in test tube **FL** is your lycopene-containing extract (impure).

8. Preparation of saturated solution of NaCl: Take approximately 20 ml water in beaker **SS** using syringe SS; then add all the solid NaCl from container **NaCl,** stir well using the glass rod. Some part of the salt may remain undissolved.
9. Use syringe **SS** to add 10 ml of saturated NaCl solution in test tube **FL** containing lycopene extract. Put the stopper on the test tube and shake gently.
10. Keep the test tube on the test tube stand. Let the liquid in the test tube separate into two distinct layers. This should take about a minute.
11. Using the plastic dropper provided**,** carefully remove most of the upper layer (coloured) into test tube **UL**.
12. Add all the anhydrous $MgSO_4$ from the container labeled **$MgSO_4$** into test tube **UL** and swirl gently to allow water to be absorbed by the salt.
13. The yellow-red coloured solution in test tube **UL** is your lycopene extract (pure).

    We shall now carry out a comparative study of absorbance between solvent and the lycopene extract.

14. Place test tube **UL** in the acrylic apparatus.
15. Using the white LED, measure the current I_l on the multimeter and record the value in **Table 1 in the answer sheet.**
16. Repeat steps 5 and 15 for Blue LED.
17. Deduce the percentage of light transmitted in each case.

Questions

If the test tube **Ab** (containing the solvent) was removed from between the photodiode and the white LED,

a) The current measured would be less than I_s
b) The current measured would be more than I_s
c) The current measured would be equal to I_s

Write the correct option in the appropriate box in the answer sheet.
Which of the following can you *deduce from your observations in the experiments* on transmitted light. Indicate your answers as YES (Y) or NO (N) on **the answer sheet**.

a) Lycopene absorbs more blue light relative to other parts of the visible spectrum.
b) Lycopene preferentially absorbs light in the red and yellow parts of the spectrum.
c) Lycopene is an antioxidant.
d) Red and yellow parts of the spectrum are absorbed relatively less compared to blue parts of the spectrum.
e) Blue light passes through the solution better compared to red light.

f) Lycopene absorbs light equally across the spectrum.

The answer sheet for this experiment is as follows.

: Extraction of lycopene from tomato **Total Marks: 6.0**

Absorbance of extract:

Q1 Observation Table 1 **[3.5 Marks]**

	Current in mA	**Blue LED**	**White LED**
1	I_s		
2	I_l		
Percentage of light transmitted			

Questions

Q2 **[1.0 Mark]**

If the test tube **Ab** (containing the solvent) was removed from between the photodiode and the white LED,

a) The current measured would be less than I_s
b) The current measured would be more than I_s
c) The current measured would be equal to I_s

Write the correct option in the box below. [ ]

Q3 **[1.5 Marks]**

The maximum absorption is in light [ ]

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Q4) [ ] [ ] [ ] [ ] [ ]

In the 2013 Olympiad 226 students carried out this experiment (in groups of 3). The total marks of this experiment were 6. The distribution of the marks received by students can be seen in Figure 4.

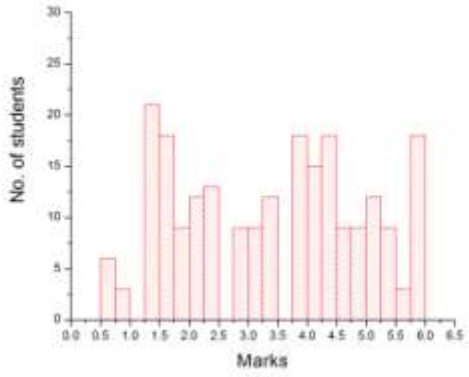

Figure 4. Number of students as a function of marks received.

This experiment is a skill oriented experiment where the ability of the student to follow the procedures are tested. It involves relatively few readings but several steps are required to be followed to prepare the solutions, set up of light-sensor, and then take the readings. Small errors in procedures can lead to wrong readings and, hence, it can be seen that out of 226 students only around 10% of the students got full marks. The distribution of marks was quite evenly spread out, reflecting a wide variation in skill levels of the students.

References:

[1]Anal Chem. 2006 Dec 15;78(24):8456-61.

[2] "A **light-emitting diode** (**LED**) is a two-lead semiconductor light source. It is a basic pn-junction diode, which emits light when activated. When a fitting voltage is applied to the leads, electrons are able to recombine with electron holes within the device, releasing energy in the form of photons. This effect is called electroluminescence, and the color of the light (corresponding to the energy of the photon) is determined by the energy band gap of the semiconductor". Ref Wikepedia.