EXERCISE 3

Body in a Lab: Aspirin Overdose

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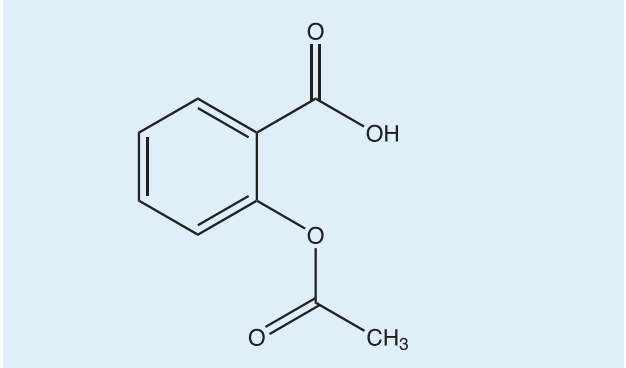
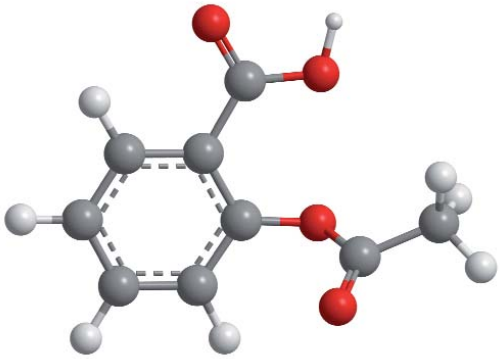
INTRODUCTION

A body has been found in the Lab! The deceased, Mr Blue, was known to be taking aspirin and a sample of his blood plasma has been sent for analysis. Use UV spectroscopy to determine the concentration of aspirin in the body and ascertain if the amount present was enough to be the cause of death.

Analysis of Salicylate in Blood Plasma by UV-Visible Spectroscopy

Aspirin or acetyl salicylic acid is a widely available drug with many useful properties. It was one of the first drugs to be commonly available and it is still widely used with approximately 35,000 tonnes produced and sold each year, equating to approximately 100 billion aspirin tablets.





Aspirin is prepared by the acetylation of salicylic acid using acetic anhydride. Its many properties as a drug include its uses as an analgesic to reduce pain, anti-inflammatory to reduce inflammation, antipyretic to reduce temperature, and platelet aggregation inhibitor to thin the blood and stop clotting.

Therapeutic levels taken after a heart attack are typically 150 – 300 mg/L and for post by-pass operations 75 mg/L. The levels of salicylate present in blood plasma can be analysed using UV-visible spectroscopy to indicate if the subject has taken a therapeutic dose or an overdose. (see following table).

Therapeutic	< 300 mg/L
Moderate Overdose	500 – 750 mg/L
Severe Overdose	>750 mg/L

Most adult deaths occur when the measured plasma level is greater than 700 mg/L. (Note: the maximum salicylate plasma levels usually occur approximately 4-6 hrs after ingestion).

METHOD

This method involves measuring the absorbance of the red-violet complex of ferric and salicylate ions at about 530 nm using a UV/Visible spectrometer.

A 5% iron (III) chloride solution has been prepared for you (5g iron (III) chloride in 100 ml of de-ionised water).

1. Preparation of Salicylate Calibration Standards

A stock solution of 2000 mg/L salicylate in 250 ml of de-ionised water has been prepared for you by dissolving 580 mg sodium salicylate in a 250 ml volumetric flask.

Make up Standard Calibration Solutions (if this has not already been done for you)

In 100 ml standard volumetric flask dilute appropriate volumes of the stock solution to give 100, 200, 300, 400, and 500 mg/L salicylate calibration standards using the dilutions given below.

2. Prepare a Blank

In a test tube prepare a blank solution by taking **1 ml** of de-ionised water and adding **4 ml of 5% iron (III) chloride** solution.

3. Prepare Standards and Unknown Plasma Sample for UV/Vis Analysis

Prepare each of the standards and the unknown plasma sample by pipetting 1 ml into a separate test tubes and adding **4 ml of 5% iron (III) chloride** solution to each (making sure each is carefully mixed).

4. Record the Absorbance

Transfer the calibration solutions, blank and unknown sample to separate cuvettes to record the absorbance. For each sample record the absorbance in the visible region between 400 – 600 nm. A peak should be observed at about 530 nm (see your demonstrator for instructions on using the UV/Visible spectrometer).

CONCENTRATION	DILUTION
100 mg/L	5 ml Stock Salicylate Solution in 100 ml De-ionised water
200 mg/L	10 ml Stock Salicylate Solution in 100 ml De-ionised water
300 mg/L	15 ml Stock Salicylate Solution in 100 ml De-ionised water
400 mg/L	20 ml Stock Salicylate Solution in 100 ml De-ionised water
500 mg/L	25 ml Stock Salicylate Solution in 100 ml De-ionised water

ANALYSIS OF RESULTS

1. Using the Beer-Lambert law plot the **absorbance versus concentration** calibration graph for the standards and using this find the unknown concentration of the salicylate present in the plasma.
2. Use this result to decide if the subject had taken a therapeutic or life threatening dose.

REFERENCES

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