DNSA reagent

Instructions for preparation and use

Version 1.0 | July 2016

Introduction

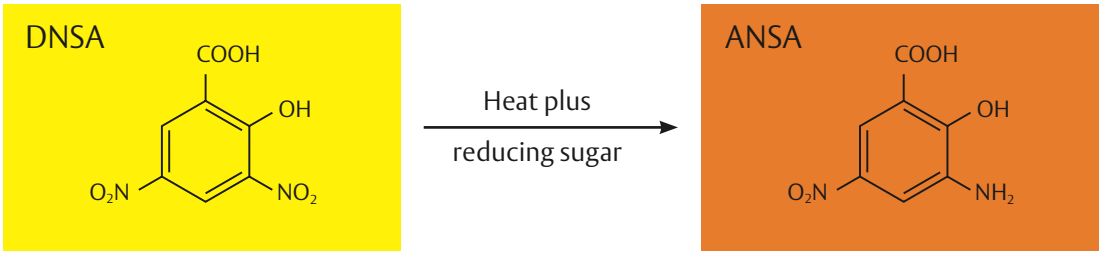
Post-16 Biology specifications in England require students to be familiar with Benedict's reagent for the detection of reducing sugars. This is in the context of '***qualitative*** *tests for the identification of biological molecules*', as specified by the subject criteria for biology (DfE, 2014).

The same DfE criteria also require students to use '*appropriate instrumentation to record* ***quantitative*** *measurements, such as a colorimeter ...*'. Most biology specifications also suggest that students carry out practical investigations of enzyme activity. It is in this latter context that we suggest that the DNSA (3,5-dinitrosalicylic acid) test, a quantitative measure of reducing sugars, is used.

DNSA is more sensitive and easier to use than Benedict's reagent. Used with a colorimeter, it is ideal for measuring the action of enzymes such as invertase, cellulase and amylase where reducing sugars are produced. Thus it helps to meet two of the important practical requirements of the current (English) biology specifications.

How it works

On heating with reducing sugars, the 3-nitro (NO_2) group of DNSA is reduced to an amino (NH_2) group.



3,5-dinitrosalicylic acid

3-amino-5-nitrosalicylic acid

The colour of the reagent changes from yellow to orange or red, depending upon the concentration of reducing sugar present. The DNSA test can detect concentrations of glucose between 0.5 mM (0.09% glucose w/v) and 40 mM (0.72% glucose w/v).

Because it is an unfamiliar product to many in the UK, this document explains how DNSA reagent should be prepared and used in the school laboratory.

Preparation

The DNSA reagent base is supplied *without* sodium hydroxide. This is because we are unable to send liquids containing sodium hydroxide in the post. You will have to add sodium hydroxide solution to the liquid supplied before it can be used. Do this as follows:

- Wear eye protection (goggles or safety glasses), protective gloves and a lab coat or apron.
- Using the 10 mL syringe supplied, add 20 mL of 2 M sodium hydroxide (NaOH) to the bottle containing the yellow-coloured DNSA mixture. *The colour of the liquid will change from opaque yellow to clear, bright orange.* [2 M NaOH contains 0.80 g of NaOH in 100 mL of solution.]
- Top up with 13 mL of distilled or deionised water to a final volume of 100 mL.
- Ensure that the bottle is closed tightly and swirl to mix the contents.
- Apply the new contents/safety label to the bottle, covering the existing label.
- If there are a few undissolved yellow lumps in the liquid, leave the bottle to stand at room temperature for an hour or so or overnight until all of the solids have dissolved.

IMPORTANT

Note that the Safety Data Sheet supplied with the product refers to the DNSA mixture *before* you have added sodium hydroxide to it. Once the sodium hydroxide has been added, the concentration of sodium hydroxide in the complete DNSA reagent is 0.4 M. This concentration of sodium hydroxide *causes skin irritation and serious eye irritation*. Protective gloves, eye protection and protective clothing *e.g.*, a lab coat or apron should be worn.

Storage

The DNSA reagent, with or without added NaOH, should be stored at room temperature. It can be stored for at least 24 months. Note that the mixture without NaOH may separate into two layers; this does not affect its performance and once NaOH has been added, the mixture is stable.

How to use it

For each sample to be tested:

- Mix 0.3 mL of DNSA reagent with 0.3 mL of the solution to be tested.
- Heat the mixture by standing it in a beaker of freshly-boiled water (*e.g.*, from a kettle) for 5–10 minutes. There is no need to boil the mixture. If reducing sugars are present, the liquid will change colour from yellow to orange or red.
- Dilute the mixture by adding 3 mL of distilled or deionised water to it.*
- Place the liquid in a cuvette and read the absorbance at 500–560 nm (using a green light or filter; the ideal wavelength to use is 540 nm).

* *If the concentration of reducing sugar in the mixture is high, the sample may need to be diluted further before the absorbance can be read in a colorimeter.*

Reference

Department for Education (April 2014) *GCE AS and A level subject content for biology, chemistry, physics and psychology*. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/446829/A_level_science_subject_content.pdf