

DETERMINING SULFIDE CONCENTRATIONS

In measuring the sulfide concentrations in our Winogradsky columns, be aware that hydrogen sulfide is quite volatile and readily oxidized. The zinc acetate, to which the sample is added, is reduced to zinc sulfide by H_2S , which is more stable in the presence of oxygen. We then add a dye solution containing N, N-dimethyl-p-phenylenediamine mono hypochloride (DPMH) and ferric chloride (a catalyst). The zinc sulfide reduces DPMH to produce methylene blue, which is responsible for the color change in your sample. We read the absorbance of the samples at 670 nm, which is a measure of the methylene blue concentration.

Reagents:

2% zinc acetate

diamine dye solution: 3.728 g n,n-dimethyl-p-phenylene diamine mono hydrochloride
6.0 g $FeCl_3 \cdot 6 H_2O$
6 N HCl

The dye solution is a strong acid. Wear a lab coat, gloves and safety glasses.

- Label 5 scintillation vials - 1 for each port of your column plus a blank (6 in total).
- Pipette 6 ml of 2% zinc acetate into each vial (may have already been done).
- Use 20 ml syringes to withdraw 1-2 ml of sample from each port, eject sample into a labeled micro-centrifuge tube and quickly SNAP TOP CLOSED (H_2S is readily oxidized and is volatile). You should wear gloves.
- Pipette 25 μ l of sample from micro-centrifuge tubes into the scintillation vials.
- Pipette 25 μ l DI into the "blank" scintillation vial.
- After this has been completed we will go to the main SES lab.
- There, add 5 ml dye solution (NASTY SO WEAR GLOVES) to the scintillation vials.
- Briefly shake the vial.
- Let stand in the dark to let the color develop. Standard incubation is 45 min (due to time constraints we may only wait 30 min.)
- Read the absorbance on the spectrophotometer at 670 nm.

Calculations:

The concentration of sulfide in the sample is calculated using a standard curve. The standard curve gives absorbance from the spectrophotometer of a series of known concentrations of sulfides in a range of concentrations likely to be found in these samples. This standard curve has been previously generated, and the slope of the standard curve is 0.542. Using this standard curve, the sulfide concentration is calculated:

$$\text{Sulfide concentration of sample} = \frac{10 \cdot (\text{Absorbance of sample} - \text{Absorbance of blank})}{0.542}$$

The number that you get from this calculation is the concentration of the sulfide in the sample in millimoles per liter or mM (millimolar).