

## 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  $\text{H}_2\text{S}$ , 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. We use smaller amount of sample (25  $\mu\text{L}$ ) because the concentration of  $\text{H}_2\text{S}$  in the columns can be quite high.

**Reagents** (previously prepare for you):

2% zinc acetate

diamine dye solution: 3.728 g n,n-dimethyl-p-phenylene diamine mono hydrochloride

6.0 g  $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$

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 (the TA may have already done this for you).
- Use 20 ml syringes to withdraw ~1 ml of sample from each port, eject sample into a labeled micro-centrifuge tube and quickly SNAP TOP CLOSED ( $\text{H}_2\text{S}$  is readily oxidized and is volatile). You can bring your column to your lab bench. You should wear gloves.
- Pipette 25  $\mu\text{l}$  of sample from each micro-centrifuge tube into the appropriately labeled scintillation vial that contains zinc acetate.
- Pipette 25  $\mu\text{l}$  DI water into the "blank" scintillation vial.
- After this has been completed we will go to the main SES lab.
- In the SES lab, add 5 mL dye solution (NASTY SO WEAR GLOVES) to the scintillation vials.
- Cap and 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 was constructed from the spectrophotometric absorbance of sulfide standards of known concentration over a range of concentrations likely to be found in your 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 as given by,

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

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