Colorimetric Determination of Nitrite in Foods

**Principle:** The sample is extracted with distilled water and the aqueous extract clarified with zinc hydroxide. Sulfanilic acid is diazotised by the nitrite and coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a pink azo dye the absorbance of which is measured at 550 nm.

![Chemical Reaction Diagrams]

**Apparatus:**

1. Waring blender or equivalent.
2. Spectrophotometer, Bausch and Lomb Spectronic 20 or equivalent.

**Reagents:**
   a) dilute 20 mL concentrated HCl to 500 mL with H₂O.
   b) mix and add 50 mL concentrated NH₄OH.
   c) dilute to 1000 mL with H₂O and mix.
   d) check pH, and adjust with HCl or NH₄OH if necessary.

2. Sodium hydroxide solution, 2% w/v in H₂O.

3. Sulfanilic acid solution, 1% in 30% acetic acid.
   a) dissolve 10 g of Sulfanilic acid in 700 mL H₂O, then add 300 mL of acetic acid and mix (Do not add acetic acid until the sulfanilic acid is dissolved).
   b) store at room temperature.

4. N-(1-naphthyl)-ethylenediamine dihydrochloride (Marshall’s Reagent), 0.1% in 60% acetic acid. Store in refrigerator; stable for 1 week.

6. Sodium nitrite standard solutions:
   I. Stock solution, 500 µg/mL.
      a) dissolve 250 mg NaNO₂ in a 500 mL volumetric flask. b) add 100 mL NH₄Cl buffer.
      c) dilute to volume with H₂O. d) mix. e) stable at 4°C for 1 week.
   II. Working solution, 5 µg/mL.
      a) transfer 1 mL stock solution to a 100 mL volumetric flask. b) dilute to volume with H₂O. c) mix. d) prepare fresh every day.

Procedure: Preparation of Standard Curve for Sodium Nitrite

1. Add 0.0, 1.0, 2.0, 4.0, 6.0 and 10.0 mL of NaNO₂ working solution to separate 50 mL volumetric flasks. Add 9.0 mL of NH₄Cl buffer and 5 mL of 60% acetic acid solution to each flask and immediately proceed to Step 2.

2. Add 5 mL of sulfanilic acid solution, 5 mL of Marshall’s reagent, dilute to volume with H₂O, mix, and let stand for 25 min in the dark. Set the spectrophotometer at 550 nm. Read standards in the cuvettes provided against blank. Prepare a calibration curve by
plotting absorbance against $\mu g$ NaNO$_2$/50 mL. The absorbance range should extend from 0 to 0.6 approx.

**E. Extraction Procedure**

Weigh ca 100g sample. Cut into very small pieces. Mix thoroughly by hand or homogenize using a blender. Weigh out a 10 g sample from above homogenate. Blend with 70 mL water and 12 mL 2% NaOH solution in blender until smooth (~5 min). Transfer slurry into a **200 mL volumetric flask**. Rinse blender with 30-50 mL H$_2$O and add to the volumetric flask. Mix well by swirling. Take out 1-2 drops of suspension from the flask and check pH with pH paper. If pH is on the slightly alkaline side heat the contents in a water bath (50°- 60°C) until the temperature of the suspension reaches close to 50°C. If the pH is less than 8 add additional amounts of 2% NaOH solution until pH rises to 8-10, and then heat as above. Occasionally swirl the contents while heating. Maintain temperature at ca 50°C for an additional 10 min, mixing occasionally.

Add 10 mL ZnSO$_4$ solution. Mix by swirling. If no white precipitate of Zn(OH)$_2$ becomes visible after the addition of ZnSO$_4$ solution add 2 to 5 mL 2% NaOH solution and mix (avoid excess addition of 2% NaOH solution).

Cool to room temperature in H$_2$O bath. Dilute to volume with H$_2$O and mix thoroughly. Filter through fluted paper (Whatman no 1 or preferably no 41) (Note 4) discarding first 20 mL filtrate, into 250 mL glass-stoppered flask. Refilter if extract is not clear.

**Determination of Nitrite**

Transfer a 10 mL aliquot of filtrate to a 50 mL volumetric flask. Add 9.0 mL NH$_4$Cl buffer and 5.0 mL of 60% acetic acid. Add 5 mL of sulfanilic acid solution and 5 mL of Marshall’s reagent. Dilute to volume with water. Mix. Let stand in dark for 25 min.