

## Analysis of starch and soluble sugars with Anthrone reagent

Anthrone dissolved in sulphuric acid may be used for the quantitative determination of different carbohydrates. Quantitative determination is only possible where the identity of sugar components is known because colour development varies with the different sugars. Nevertheless, the anthrone method is widely used for the determination of starch and soluble sugars in plant material.

Generally sugars and carbohydrates are extracted from dried and ground plant material. First soluble sugars are extracted with aqueous ethanol, later starch is extracted with an acid. Acidic starch extracts are typically clear, however ethanolic sugar extracts may be green (leaves) or brown (roots). To remove these interfering colours first pre-extract plant material with 100% acetone (Marshall 1986, Oren). Any one of hundreds of extraction procedures may be used to extract soluble sugars not just aqueous ethanol. If methanol/chloroform/water was used it would not be necessary to do first an acetone extraction.

### References

Hansen J, Møller IB (1975) *Anal Biochem* 68: 87-94.

Oren et al. *Oecologia* (????) 75: 28-29

Marshall (1986) *Plant and Soil* 52-54

### Extraction procedure

1. Dry and grind leaf material
2. Weigh 10 -20 mg of ground leaf into a centrifuge tube or similar
3. Extract interfering pigments with 100% acetone, e.g using ultra-turrax and filtering/centrifuging
4. Extract sugars with 2 \* 2.5-mL aliquots of 80% ethanol
5. Filter or centrifuge and keep supernatant for soluble sugar analysis
6. To the residue add 5 mL of 1.1% HCl
7. Heat in a water bath at 100°C for 30 min
8. Dilute to 10 mL with DI water

### Analysis procedure

1. Turn on spectrophotometer and let warm up
2. Make Anthrone reagent: dissolve 1 g of anthrone in 500 mL of 72% sulphuric acid
3. Pipette 1.0 mL of test solution into a 10-mL test tube and cool to 0°C on ice
4. Add 5 mL of ice-cold anthrone reagent. Note: anthrone reagent is quite viscous, particularly when cold
5. Heat for **exactly** 11 minutes at 100°C (in water bath) and cool rapidly to 0°C on ice
6. Read  $A_{630}$  (against water) within an hour

Carry standard starch solutions (0 to 10 mg starch/10 mL solution) and a blank through extraction and analysis procedure.

**Note:** starch is normally stored in the fridge (0-5°C) to minimise degradation/hydrolysis.