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Estimation of Glycogen Content

Experiment 13 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To estimate glycogen in an instructor-prepared extract using a calibrated carbohydrate colour method.

Principle

Acidic anthrone chemistry produces a coloured response from carbohydrates. A glucose-calibrated assay can estimate glycogen after appropriate extraction and hydrolysis, but it is not specific to glycogen when other carbohydrates remain. Glucose-equivalent mass is converted to anhydroglucose mass using 162/180 = 0.90.

Requirements

- Commercial glycogen standard or instructor-prepared purified teaching extract; glucose calibration series.
- Instructor-prepared anthrone reagent, matching tubes, ice bath, controlled heating bath, spectrophotometer near 620 nm and protective equipment.

Procedure

1. Prepare the extract

Use the instructor-provided extract and record its original material mass, final extract volume and prior dilutions. This activity does not require animal dissection.

2. Calibration

Prepare the laboratory-approved glucose mass series, blank and quality-control glycogen portion. Keep the aqueous volume identical in all tubes.

3. Colour development

Under direct supervision, add the prepared acidic anthrone reagent using the prescribed order and cooling precautions. Apply identical heating and cooling times to every tube.

4. Read

Measure near 620 nm at the method-specified interval. Plot blank-corrected absorbance against glucose mass and use only the linear interval.

5. Calculate

Find glucose-equivalent mass in the assay aliquot, apply 0.90 to obtain glycogen-equivalent mass, then scale by extract volume / aliquot volume and any prior dilution.

6. Interpret

Report the extraction and calibration basis. If interfering carbohydrate was not removed, identify the result as apparent glycogen-equivalent carbohydrate rather than a specific pure-glycogen assay.

Observations and Record

Record	Entry
Original material mass / extract volume	_____
Calibration masses / absorbances	_____
Aliquot volume / prior dilution	_____
Glucose-equivalent mass / corrected glycogen result	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- A 0.10 mL aliquot corresponds to 40 µg glucose equivalents. Glycogen in that aliquot = 40 × 0.90 = 36 µg.
- For 10.0 mL extract with no earlier dilution: total = 36 × 10.0 / 0.10 = 3600 µg = 3.60 mg. If original material was 0.50 g, content = 7.20 mg/g.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Strong-acid reagent addition and heating are supervised. Never add water rapidly to concentrated acid or heat a closed tube.
- Use identical colour-development conditions and correct all aliquot/dilution factors.
- Do not claim assay specificity merely from a positive anthrone colour.

Viva Questions and Answers

1. Why multiply glucose equivalents by 0.90?

A glycogen anhydroglucose unit has mass 162 relative to free glucose at 180.

2. Why use a calibration curve?

It determines the colour response under the actual assay conditions.

3. What can inflate an apparent glycogen result?

Other carbohydrate present in the extract.

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