A 18 b - Total Lactose in Milk and Whey Powders by Gravimetric

GEA Niro Method No. A 18 b
Revised: June 2006

1. **Principle**
   The method is based on lactose reducing a cupric salt complex to Cu2O under specified conditions. The amount of Cu2O precipitated is proportional to the lactose content.

2. **Scope**
   The method is used on all milk and whey powders.

3. **Apparatus**
   1. 500 ml volumetric flask.
   2. 2 x 500 ml brown glass bottles.
   3. Pipettes - 25 and 50 ml.
   4. 400 ml graduated beaker.
   5. Bunsen burner or hot plate.
   6. Wire gauze with a ceramic centre.
   7. Filter paper.
   8. Glass funnel.
   9. Stop watch.
   10. Watch glass.
   13. Disposable weighing dish
   14. 10 and 25 ml graduated cylinders.
   15. Vacuum pump.
   16. Analytical balance, capable of weighing 0.1 mg.
   17. Desiccator.
   18. Drying oven without forced air circulation, and with a thermostatic control capable of maintaining the temperature at 100°C ± 1°C.

4. **Chemicals**
   1. CuSO₄·5H₂O, p.a., Merck.
      Copper(II)sulfate: R 22 and S 24.
   2. C₃H₄Na₂O₆·H₂O, p.a., Merck.
      Potassium sodium tartrate.
   2. NaOH, p.a., Merck.
      Sodium hydroxide pellets: R 35 and S 2-26-37/39.
   3. KOH, p.a., Merck.
      Potassium hydroxide, pellets: R 35 and S 2-26-37/39.
   4. 96% Ethanol: R 11 and S 7-16.
      R ≈ DK risk sentences
      S ≈ DK safety sentences
5. **Reagents**

1. **Fehling I.**
   - Copper(II)sulphate solution.
   - Dissolve 34.639 g CuSO₄, 5H₂O in deionized water.
   - Dilute to 500.0 ml in a volumetric flask, and filter through a paper filter.
   - Store in a 500 ml brown glass bottle.

2. **Fehling II.**
   - Alkaline tartrate solution.
   - Dissolve 173.0 ± 0.1 g C₄H₆Na₂O₆, H₂O and 50.0 ± 0.1 g NaOH pellets in deionized water and dilute to 500 ml in a volumetric flask.
   - Allow to stand for 2 days, before filtering through a paper filter.
   - Store in a 500 ml brown glass bottle.

3. **Potassium hydroxide solution.**
   - Dissolve 15.567 g KOH in deionized water. Dilute to 500.0 ml in a volumetric flask.

6. **Procedure**

1. Weigh out according to type of powder ± 0.05 g:
   - whey 1.60 g
   - skim milk 2.00 g
   - whole milk 2.80 g
2. Dissolve the powder in approx. 200 ml 60°C deionized water in a 500 ml volumetric flask. Invert the solution until all powder is dissolved, and cool to 20°C.
3. Add 10 ml of Fehling I solution and 7.5 ml (measuring pipette) KOH solution (the solution must still be acidic, check with pH paper), and dilute to 500.0 ml.
4. Mix carefully and filter through a dry filter.
5. Pipette 25.0 ml of Fehling I solution and 25.0 ml of Fehling II solution into a 400 ml beaker.
6. Add 25 ml of the filtrate (F4) and 25 ml of deionized water.
7. Cover the beaker with a watch glass and heat it over a Bunsen burner or a hot plate. The heat must be regulated so boiling begins after 4 minutes. Continue boiling for exactly 2 minutes. It is important that these regulations are strictly maintained. For this purpose it is recommended to make a preliminary test, using 50 ml deionized water and 50 ml reagent.
8. Filter the solution immediately through a dried and weighed glass filter crucible by means of suction.
9. Transfer the precipitated Cu₂O quantitatively to the glass filter crucible, and wash it carefully, first with 60°C deionized water, then with 10 ml alcohol and finally with 10 ml of ether.
10. Dry the precipitate in an oven at 100°C for 30 minutes, cool in a desiccator and weigh.
11. Carry out a blank test according to F5-F10 using deionized water instead of reducing sugar filtrate. If the weight of the Cu₂O obtained in the blank is more than 0.5 mg, correct the results of reducing sugar determination accordingly.

7. **Result**

   Use the Hammond Table to express the weight of lactose equivalent to the weight of Cu₂O.

   \[
   \% \text{ lactose in powder} = \frac{A \times 500 \times 100}{W \times ml \times 1000}
   \]

   \(A\) = mg lactose equivalent to the weight of Cu₂O as found in the table.
   \(W\) = weight of milk powder
   \(ml\) = ml filtrate taken with pipette

8. **Reference**

   GEA Niro Research Laboratory
Hammond table for calculating lactose values
Expressed in mg

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