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## Application Note

### THE DETERMINATION OF CALCIUM IN BIOLOGICAL FLUIDS

#### Introduction

The Flame Photometer may be used for the determination of calcium in blood serum, cerebrospinal fluid, urine, bile, oedema fluid, etc. This determination, although not so simple as the flame photometric determination of potassium or sodium, still gives a certain saving in time over the chemical method since there is no need for repeated washing due to the fact that the ammonium salts do not affect the flame photometric reading.

#### Principle

The calcium is precipitated with an oxalic acid-ammonium oxalate mixture, which has a fixed pH so as to act as a buffer, mainly for urine determination where magnesium might otherwise be precipitated. The solution is centrifuged and the supernatant fluid decanted off to be used in sodium and potassium determinations, if required. The precipitate is then dissolved in perchloric acid, which is sufficiently concentrated to dissolve the precipitate but not concentrated enough to lower the calcium reading. This solution is used in the Flame Photometer.

It should be noted that with heparinized blood plasma, the calcium should be precipitated promptly otherwise particles of fibrin may form and spin down with the precipitate. They then tend to block the atomiser and also cause the oxalate precipitate to lose its characteristic crystalline form and appear instead as a kind of protein oxalate complex, which is extremely difficult to dissolve. If it is necessary to use heparinized plasma it is, therefore, advisable to remove proteins before precipitating the oxalate.

#### Reagents

1. 0.1M Oxalic Acid (12.6g Oxalic Acid dihydrate per liter)
2. 0.1M Ammonium Oxalate (14.2g Ammonium Oxalate mono hydrate per liter)
3. Perchloric Acid 60%
4. Calcium chloride (anhydrous)
5. Deionized Water

#### Preparation of Solutions

1. Ammonium oxalate/oxalic acid mixture
  - a) 5ml of 0.1M oxalic acid
  - b) 95ml of 0.1M ammonium oxalate
2. Perchloric Acid – 0.05N, freshly prepared each day
  - a) 1.66ml of 60% perchloric acid
  - b) Dilute to 200ml with deionized water
3. Calcium chloride solutions
  - a) Stock – 13.9g of calcium chloride dissolved in deionized water and made up to 1000ml. The concentration needs to be checked by a chloride standard estimation.
  - b) Daily Standard – Dilute stock solution 1 in 100 with 0.05N perchloric acid.

This Method is meant for your guidance. Techne and Jenway are not responsible for the recommendations in detail.  
Method Ref. Flame Calcium in Biological Fluids  
May 2006/ REV B





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The concentration of this solution is 5mg Calcium/100ml.

### **Sample Preparation**

1. Pipette 2ml of serum into a rounded centrifuge tube and add 3ml of the ammonium oxalate/oxalic acid mixture.
2. Mix and allow to stand for 30 minutes. A precipitate will form.
3. Centrifuge well and decant off the supernatant fluid, then drain.
4. Add 4ml of 0.05N perchloric acid.
5. Shake vigorously to dissolve the precipitate.

### **Preparation of a Control**

1. Dilute the stock solution 1 in 50 with 0.05N perchloric acid. The concentration of this solution is 10mg Calcium/100ml.

### **Procedure**

1. Set up the flame photometer as described in the instruction manual.
2. Set the flame photometer to the Calcium filter position.
3. Spray deionized water and adjust the zero control until the meter reads zero.
4. Spray the daily standard and adjust reading to 50.
5. Repeat steps 3 and 4 until no further adjustment is necessary.
6. Spray the control and note the reading.
7. Spray the sample and note the reading.
8. Calculate the concentrations of the control and sample using the following:

$$\text{Calcium content in mg per 100ml} = \frac{\text{Reading}}{5}$$

$$\text{Calcium content in mEq per Liter} = \frac{\text{Reading}}{10}$$

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