Determination of analytes in whole blood samples

Concentrated Carrez I solution: 200 mL

Dissolve 30 g of potassium hexacyanoferrate (II) {$K_4[Fe(CN)_6.3H_20$} (Sigma cat. no. P-9387) in 200 mL of distilled water. Store at room temperature.

Concentrated Carrez II solution: 200 mL

Dissolve 60 g of zinc sulphate {$ZnS0_4.7H_20$} (Sigma cat. no. Z-4750) in 200 mL of distilled water. Store at room temperature.

Procedure:

Heat 1 mL of whole blood sample at approx. 80 °C for 20 min in a microfuge tube then centrifuge at 13000 x g for 10 min and recover the supernatant. Add 20 uL Carrez Reagent II and mix thoroughly, then add 20 uL Carrez Reagent I and mix thoroughly. Centrifuge the sample again at 13000 x g for 10 min and recover the clarified supernatant for use in the assay. If required, dilute the sample appropriately in distilled water for the assay.

Note: The final volume of the clarified supernatant will be approximately one quarter of the starting volume of the original sample. Therefore adjust the volume of the staring material as required to obtain sufficient volume of clarified sample for the test.

Determination of analytes in biological tissue samples

1 M Perchloric acid:

(Sigma Cat No. 244252; MW 100.46; d = 1.664 (g/mL); 70% assay; 11.59 M)
Add 8.6mL perchloric acid to 92.4 mL of distilled water and mix thoroughly.

1 M Potassium hydroxide:

(Sigma Cat No. 60369; MW 56.11; 86% assay)
Add 6.5 g of potassium hydroxide pellets to approximately 80 mL of distilled water and stir to dissolve. Make to 100 mL with distilled water.

Procedure:

Accurately weigh approx. 5 g of representative biological tissue into a 100 mL Duran® bottle. Add 20 mL of 1 M perchloric acid and homogenise for 2 min using a Ultraturrax® or Polytron® homogeniser (or equivalent). Quantitatively transfer to a 40 mL glass beaker and adjust the pH to approx. 7.0 - 8.0 using 1 M KOH. Quantitatively transfer to a 100 mL volumetric flask and adjust to the mark with distilled water (ensuring the fat containing layer is "above" the mark, and the aqueous layer is "at" the mark). Store on ice for 20 min to precipitate potassium perchlorate and allow separation of the fat (if present). Centrifuge an appropriate volume of the sample at 13000 x g for 10 min and recover the clarified supernatant for use in the assay, alternatively filter through Whatman No. 1 filter paper, discarding the first 3-5 mL, and use the clear filtrate for the assay. If required, dilute the sample appropriately in distilled water for the assay.

Note: The amount of starting material and volumes used can be adjusted accordingly depending on the amount of analyte present in the sample.

Determination of analytes in biological fluid samples (e.g. urine and serum)

For some biological fluid samples it may be sufficient to test them directly without any sample preparation other than appropriate dilution in distilled water. If this is not adequate then deproteinisation with either perchloric acid or trichloracetic acid may be required.

1M Perchloric acid:

(Sigma Cat No. 244252; MW 100.46; d = 1.664 (g/mL); 70% assay; 11.59 M)
Add 8.6mL perchloric acid to 92.4 mL of distilled water and mix thoroughly.

1M Potassium hydroxide:

(Sigma Cat No. 60369; MW 56.11; 86% assay)
Add 6.5 g of potassium hydroxide pellets to approximately 80 mL of distilled water and stir to dissolve. Make to 100 mL with distilled water.

Procedure:

Deproteinise biological samples by adding an equal volume of ice-cold 1 M perchloric acid with mixing. Adjust the pH to approx. 7.0 – 8.0 using 1 M KOH. Centrifuge an appropriate volume of the sample at 1500 x g for 10 min and recover the supernatant for use in the assay, alternatively filter through Whatman No. 1 filter paper, discarding the first 3-5 mL, and use the filtrate for the assay. If required, dilute the sample appropriately in distilled water for the assay.

Alternatively, use 50 % (w/v) trichloroacetic acid instead of perchloric acid.