1. **Purpose**

1.1. This procedure is to ensure that Dextran Sulphate 40,000 MW powder manufactured at Dextran Products is subject to full tests following the test methods in the QC lab.

1.2. This procedure is to ensure that the quality of Dextran Sulphate 40,000 MW powder meets the specifications.

2. **Scope**

2.1 This procedure applies to Dextran Sulphate 40,000 MW powder produced and marketed by Dextran Products, for both Final QC and Long-Term & Short-Term Stability Study testing.

2.2 This procedure applies to QC Analytical Chemists for performing all testing and to QA manager for reviewing all documentation and releasing the batches.

3. **Regulatory Basis**

3.1. The cGMPs state that no lot or batch of product shall be available for sale unless it complies with the specifications for that product.

4. **Responsibility**

4.1. QC Analytical Chemists are responsible for performing all testing of final QC and Long-Term & Short-Term Stability study according to established test methods and procedures.

4.2. The QC Manager is responsible for ensuring that full tests of Dextran Sulphate 40,000 MW powder are completed, and for releasing all data, which are generated in the QC lab and meet the required specifications.

4.3. The QA Manager is responsible for reviewing all **Batch Production Record (BPRs)**, all QC testing data and documentation to final release of the products.

5. **Procedures**

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5.1 APPEARANCE

Sample
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)

Equipment
Transparent Plastic Bag

5.1.1 Performing and Observing Unknown Powder Sample

5.1.1.1 After spray drying, Dextran Sulphate powder sample (**DS40**) is packed in a transparent plastic bag.

5.1.1.2 Carefully observe the appearance of the powder in the bag from different sides.

5.1.2 Expected Results

5.1.2.1 DS40 powder sample is a white to light yellow powder.

5.1.2.2 Record the result in the **DS** notebook.

5.2 LOSS ON DRYING (LOD)

Sample
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Laboratory Oven set at **105 ± 5°C**
Calibrated Thermometer
Desiccator with Silica Gel
Spatula
Glass-stoppered Shallow Weighing Bottle
Petri Dish
Permanent Marker
Pair of Crucible Tongs
Pair of Cotton Gloves

- **Prepare each sample in duplicate.**

5.2.1 Preparing Unknown Powder Samples

5.2.1.1 Label two glass-stoppered shallow weighing bottles to be used with a permanent marker.

5.2.1.2 Using a pair of crucible tongs, weigh the weighing bottles and record the weights in the **DS** notebook (**Wt. 1**).

5.2.1.3 Using a spatula, accurately weigh about **1.0000g to 1.5000g** of Dextran Sulphate powder sample in each pre-weighed weighing bottle.

5.2.1.4 Record the total weights of the sample and the weighing bottle in the **DS** notebook (**Wt.2**).

5.2.2 Drying Powder Samples

5.2.2.1 Using a pair of crucible tongs, place the sample weighing bottles in a petri dish.

5.2.2.2 Check and ensure that the Laboratory oven is at the required temperature, set at **105°C**.

5.2.2.3 Wear a pair of cotton gloves, open the oven door and carefully place the petri dish containing the sample weighing bottles into the oven. Make sure the glass stopper is open.

5.2.2.4 Close the oven door and heat the powder sample for **about 4 to 5 hours**.

5.2.2.5 When the time is up, wearing a pair of cotton gloves, close the glass stoppers on the weighing bottles by using a pair of crucible tongs and cautiously take out the Petri dish from the oven.

5.2.2.6 Immediately place the petri dish into the desiccator for **about 5 minutes** to allow it to reach room temperature.

5.2.2.7 Using a pair of crucible tongs, take out and re-weigh the weighing bottles containing the unknown powder sample.

5.2.2.8 Record the weights after heating in the **DS** notebook **(Wt. 3)**.

- **Dried Dextran Sulphate powder is hygroscopic**.

5.2.2.9 Determine percentage **(w/w)** of Loss on Drying in Dextran Sulphate powder samples from the difference of the weights before drying and after drying. The results are calculated by following the equation in step **5.2.3.**

5.2.3 Calculations

5.2.3.1 *Calculation of Loss on Drying*

$$\textbf{Loss On Drying \% (w/w)} = \frac{\textbf{(Wt. 2 – Wt. 3)}}{\textbf{(Wt. 2 – Wt. 1)}} \times 100\%$$

Where:

- Wt. 1 = Weight of empty weighing bottle (g)
- Wt. 2 = Weight before heating: weighing bottle + sample (g)
- Wt. 3 = Weight after heating: weighing bottle + sample (g)
- 100% = Result unit conversion from g to % (w/w)

5.2.3.2 *Calculation of Net Sample Weight on Dry Basis*

5.2.3.2.1 The net weight of Dextran Sulphate Powder sample is calculated based on the Loss on Drying (**LOD**) data, which is used for the result calculation.

Net Sample Weight on a Dry Basis = Sample Weight (g) - LOD

= Sample Weight (g) x (100% - LOD%)

= Sample Weight (g) x Sample Assay%

e.g. Net Sample Weight on Dry Basis (g) = Sample Weight x [100% – LOD% (4.0%)]

= 5.0 x 96.0% = 4.8

5.3 IDENTIFICATION (Test for Acrinol, Sulphate, and Dextran)

Sample, Reagents and Solution
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)
Distilled Water
Acrinol Powder
Concentrated Hydrochloric Acid (HCl, 36.5 ~ 38%)
Sodium Hydroxide (NaOH) Powder
Barium Chloride Dihydrate ($BaCl_2 \bullet 2H_2O$) Powder
Anthrone Powder
Concentrated Sulphuric Acid (H_2SO_4, 95% ~ 98%)
Glacial Acetic Acid (99.7%)

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated Analytical Balance, A and D EJ-610 or equivalent (0.00)
Micro Pipette 1-ml, 0.5ml~5-ml (adjustable)
Micro Pipette Tips
Hot / Stirring Plate
Magnetic Stirring Bar
Boiling Water Bath

Glassware and Accessories
Spatula
Weighing Paper / Boat
Graduated Cylinder, 50-ml, 100-ml
Volumetric Flask with stopper, 100-ml, 1000-ml
Beaker, 100-ml, 250-ml
Erlenmeyer/Conical Flask, 250-ml
Unitary Wash Bottle
Pasteur Pipette
Rubber Bulb
Test Tubes
Labels
Parafilm

I. Test for Acrinol Identification

5.3.1 Preparing 1 % Acrinol Solution

5.3.1.1 Using a spatula, accurately weigh about **1.00g** of Acrinol powder on a piece of weighing paper. Then transfer it into a 100-ml beaker.

5.3.1.2 Record the reagent powder weight in the Lab Reagent notebook.

5.3.1.3 Rinse the weighing paper with distilled water and add the rinsings into the beaker.

5.3.1.4 Continue adding about 50ml of distilled water into the 100-ml beaker.

5.3.1.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.3.1.6 Transfer the solution into a 100-ml volumetric flask.

5.3.1.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask.

5.3.1.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.3.1.9 Transfer the final resulting solution into a storage bottle and label it.

5.3.2 Preparing Diluted Hydrochloric Acid (10% HCl, USP)

5.3.2.1 Using a 100-ml graduated cylinder, measure approximately **60ml** of distilled water and transfer it into a 100-ml volumetric flask.

5.3.2.2 Using a 50-ml graduated cylinder, measure about **22.6 ml** of **concentrated HCl, 36.5% ~38%,** solution and cautiously add it into the 100-ml volumetric flask.

5.3.2.3 Dilute the solution with distilled water up to volume. Mix it well on a stirring plate with a magnetic stirring bar.

5.3.2.4 Transfer the resulting solution into a storage bottle and label it.

5.3.3 Preparing 2N NaOH Solution

5.3.3.1 Measure about **100ml** of distilled water in a 250-ml beaker.

5.3.3.2 Using a spatula, accurately weigh about **80.00g** of Sodium Hydroxide crystals in a weighing boat. Then cautiously add it to the 250-ml beaker.

5.3.3.3 Record the reagent powder weight in the Lab Reagent notebook.

5.3.3.4 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.3.3.5 Continue adding about 100 ml of distilled water into the beaker.

5.3.3.6 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.3.3.7 Transfer the solution into a 1000-ml volumetric flask.

5.3.3.8 Rinse the original beaker with distilled water and add the rinsings into the 1000-ml volumetric flask.

5.3.3.9 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.3.3.10 Transfer the final resulting solution into a storage bottle and label it.

5.3.4 Preparing 5 % Dextran Sulphate Sample Stock Solution (5% D.S.S)

- **The sample weight used for the result calculation is on a Dry Basis.**

5.3.4.1 Using a spatula, accurately weigh about **5.0000g** of Dextran Sulphate powder sample in a weighing boat. Then transfer it into a 100-ml beaker.

5.3.4.2 Record the sample powder weight in the **DS** notebook.

5.3.4.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.3.4.4 Continue adding about 50ml of distilled water into the 100-ml beaker.

5.3.4.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.3.4.6 Transfer the solution into a 100-ml volumetric flask.

5.3.4.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask.

5.3.4.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.3.4.9 Label the final resulting solution as **5% D.S.S**.

5.3.5 Identifying Unknown Powder Samples

5.3.5.1 Prepare two clean glass test tubes.

5.3.5.2 Pipette **1.0 ml** of **1%Acrinol** solution into each test tube separately.

5.3.5.3 Add **5.0 ml** of **5% D.S.S** solution prepared in **step 5.3.4** into each tube, respectively.

5.3.5.4 Mix both tubes well by gentle hand shaking.

5.3.5.5 Observe that a yellow flocculent precipitate is formed in both tubes.

5.3.5.6 Using a Pasteur pipette, add a few drops of **10% HCl** solution into one test tube.

5.3.5.7 Using a Pasteur pipette, add a few drops of **2N NaOH** solution into the other test tube.

5.3.5.8 Mix both tubes well by hand shaking again and observe the sample reactions.

5.3.6 Expected Result

5.3.6.1 The yellow flocculent precipitate is almost insoluble either in acid or in alkali.

5.3.6.2 Record the result in the **DS** notebook.

II. Test for Sulphate Identification

5.3.7 Preparing 10% Barium Chloride ($BaCl_2$) Solution

5.3.7.1 Using a spatula, accurately weigh about **10.00g** of Barium Chloride dihydrate powder in a weighing boat. Then transfer it into a 100-ml beaker.

5.3.7.2 Record the reagent powder weight in the Lab Reagent notebook.

5.3.7.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.3.7.4 Continue adding about 50ml of distilled water into the 100-ml beaker.

5.3.7.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.3.7.6 Transfer the solution into a 100-ml volumetric flask.

5.3.7.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask.

5.3.7.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.3.7.9 Transfer the final resulting solution into a storage bottle and label it.

5.3.8 Preparing and Identifying Unknown Powder Samples

5.3.8.1 Pipette **10ml** of distilled water into a 100-ml beaker.

5.3.8.2 Using a suitable graduated cylinder, measure **10ml** of **concentrated HCl** solution and cautiously, slowly add it into the beaker.

5.3.8.3 Place the beaker on a hot plate and mix the solution with a magnetic stirring bar.

5.3.8.4 Using a spatula, accurately weigh about **1.00g** of Dextran Sulphate powder sample in a weighing boat. Then cautiously transfer it into the acid beaker.

5.3.8.5 Record the sample powder weight in the **DS** notebook.

5.3.8.6 Heat the beaker to boiling with continuing mixing for **2 minutes**. Then cool the resulting solution down to room temperature.

5.3.8.7 Using a Pasteur pipette, add a few drops of **10% $BaCl_2$** solution into the beaker and observe the sample reaction.

5.3.9 Expected Result

5.3.9.1 A heavy precipitate of **Barium Sulphate ($BaSO_4$)** is formed.

5.3.9.2 Record the result in the **DS** notebook

III. Test for Dextran Identification

5.3.10 Preparing Anthrone solution in 90 ~ 93% Sulphuric Acid (H_2SO_4)

- ***The Anthrone Solution is sensitive to oxygen. Prepare it fresh on the day of use.***

5.3.10.1 Pipette **10ml** of distilled water into a 250-ml erlenmeyer flask.

5.3.10.2 Measure **190ml** of Concentrated **Sulphuric Acid (H_2SO_4)** with a graduated cylinder and slowly add it into the erlenmeyer flask.

5.3.10.3 Place the erlenmeyer flask on a stirring plate and mix the diluted acid solution well with a magnetic stirring bar, until it reaches room temperature.

5.3.10.4 Accurately weigh about **400mg (0.4000g)** of Anthrone powder on a piece of weighing paper.

5.3.10.5 Record the reagent powder weight in the Lab Reagent notebook.

5.3.10.6 Quickly and cautiously transfer Anthrone powder into the erlenmeyer flask while the diluted H_2SO_4 solution is mixing.

5.3.10.7 Rinse the weighing paper with a few drops of distilled water, add the rinsings into the erlenmeyer flask as well, and cover entire erlenmeyer flask with a piece of aluminum foil.

5.3.10.8 Continue mixing the resulting solution until Anthrone powder is completely dissolved and then cool it down to room temperature.

- Complete the preparation **within 30 minutes** due to oxygen sensitivity of Anthrone powder.

5.3.10.9 Transfer Anthrone solution into a brown bottle with a bottle-top dispenser and place it in the freezer until the time of use.

5.3.11 Preparing 1% Dextran Sulphate Sample Stock Solutions (1% D.S.S)

➢ **The sample weight used for the result calculation is on a Dry Basis.**

5.3.11.1 Using a spatula, accurately weigh about **1.0000g** of Dextran Sulphate powder sample in a piece of weighing paper. Then transfer it into a 100-ml beaker.

5.3.11.2 Record the sample powder weight in the **DS** notebook.

5.3.11.3 Rinse the weighing paper with distilled water and add the rinsings into the beaker.

5.3.11.4 Continue adding about 50ml of distilled water into the 100-ml beaker.

5.3.11.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.3.11.6 Transfer the solution into a 100-ml volumetric flask.

5.3.11.7 Rinse the original beaker with the distilled water and add the rinsings into the volumetric flask as well.

5.3.11.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.3.11.9 Label the final resulting solution as **1% D.S.S**.

5.3.12 Identifying Unknown Powder Samples

5.3.12.1 Take the cold Anthrone Solution out from the freezer.

5.3.12.2 Pipette **1.0ml** of **1% D.S.S** solution prepared in **step 5.3.11** into a test tube.

5.3.12.3 Pipette **5.0ml** of **Anthrone Solution in 90 ~ 93% H_2SO_4** solution prepared in **step 5.3.10** into the same test tube. Then mix the tube well by hand shaking

5.3.12.4 Heat the tube in a boiling water bath for **10 minutes**.

5.3.12.5 Observe that the solution turns green and then blue green color.

5.3.12.6 Using a Pasteur pipette, add a few drops of **99.7% Glacial Acetic Acid** solution into the test tube.

5.3.13 Expected Result

5.3.13.1 The blue green color does not change with the addition of Glacial Acetic Acid.

5.3.13.2 Record the result in the **DS** notebook.

5.4 FREE INORGANIC SULPHATE

Sample, Reagents and Solutions

1% Dextran Sulphate Sample Stock Solution (**1% D.S.S** from step ***5.3.11***)
Distilled Water
Concentrated Hydrochloric Acid (HCl, 36.5 ~ 38%)
Diluted HCL (10% HCl, USP) (The same as step ***5.3.2***)
Barium Chloride Dihydrate ($BaCl_2 \bullet 2H_2O$) Powder
Sodium Sulphate, Anhydrous (Na_2SO_4, ACS Grade)

Equipment and Accessories

Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated Spectrophotometer, UV-2101PC, Shimadzu or Equivalent
Spectrophotometer Cuvettes
Laboratory Oven set at 105°C +/-5°C
Calibrated Thermometer
Desiccator with Silica Gel
Micro-Pipette, 0.5-ml ~ 5-ml (Adjustable)
Micro-Pipette Tips
Stirring Plate
Magnetic Stirring Bar

Glassware and Accessories

Spatula
Weighing Boat/Paper
Volumetric Flasks with stopper, 100-ml, 500-ml, 1000-ml
Beaker, 100-ml
Graduated Cylinder, 50-ml, 100-ml
Glass-Stoppered Weighing Bottle
Pair of Crucible Tongs
Pair of heat resistant gloves
Test Tubes
Utility Wipers
Labels, Permanent Marker

5.4.1 Preparing Sulphate Standard Solution (0.2% SO_4^{-2}Solution)

5.4.1.1 Weigh about **2.0000g** of Sodium Sulphate Anhydrous powder in a labelled glass-stoppered weighing bottle, using a pair of crucible tongs place it in the oven set at **105°C**. Leave the stopper open and dry for about **4 to 5 hours**.
NOTE: Sodium Sulfate Anhydrous is very hygroscopic. The drying step is required before making a standard solution.

5.4.1.2 After drying, using a pair of crucible tongs replace the glass stopper and then place the weighing bottle into the desiccator for about **5 minutes** to allow it to reach room temperature.

5.4.1.3 Weigh **1.479g** of dried Sodium Sulphate Anhydrous powder on weighing paper and transfer to a 500-ml volumetric flask. Rinse the weighing paper with distilled water and add the rinsings to the volumetric flask.

5.4.1.4 Record the standard weight in the lab reagent notebook.

5.4.1.5 Add about 300-ml of distilled water into the volumetric flask and mix on a magnetic stirring plate until the powder is completely dissolved. Dilute the solution with distilled water to volume.

5.4.1.6 Transfer the final solution into a storage bottle and label it as **0.2% SO_4^{-2}** Solution.

5.4.2 Preparing Diluted Hydrochloric Acid (10% HCl, USP)

5.4.2.1 Using a 100-ml graduated cylinder, measure approximately **60ml** of distilled water and transfer it into a 100-ml volumetric flask.

5.4.2.2 Using a 50-ml graduated cylinder, measure about **22.6ml** of **concentrated HCl, 36.5% ~ 38%,** solution and cautiously add it into the 100-ml volumetric flask.

5.4.2.3 Dilute the solution with distilled water up to volume. Mix it well on a stirring plate with a magnetic stirring bar.

5.4.2.4 Transfer the resulting solution into a storage bottle and label it.

5.4.3 Preparing 0.1M Barium Chloride ($BaCl_2$) Solution

5.4.3.1 Using a spatula, accurately weigh about **2.4426g** of Barium Chloride Dihydrate Powder in a weighing boat. Then transfer it into a 100-ml beaker.

5.4.3.2 Record the reagent powder weight in the Lab Reagent notebook.

5.4.3.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.4.3.4 Continue adding about 50ml of distilled water into the 100-ml beaker as well.

5.4.3.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.4.3.6 Transfer the solution into a 100-ml volumetric flask.

5.4.3.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask.

5.4.3.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with the magnetic stirring bar.

5.4.3.9 Transfer the final resulting solution into a storage bottle and label it.

5.4.4 Detecting Free Inorganic Sulphate in sample solution against Sulphate Standard Solution

5.4.4.1 Prepare three test tubes, and label them as **Blank**, **Sulphate Standard (0.2%)**, and **Unknown Sample**, respectively.

5.4.4.2 Pipette **5 ml** of distilled water into **Blank** test tube.

5.4.4.3 Pipette **5 ml** of 0.2 % Sulphate Standard Solution (**0.2% S.S** prepared in step ***5.4.1***) into **Sulphate Standard (0.2%)** test tube.

5.4.4.4 Pipette **5ml** of 1% Dextran Sulphate Stock Solution (**1%D.S.S** prepared in step ***5.3.11***) into **Unknown Sample** test tube.

5.4.4.5 Add **0.5ml** of diluted **10% HCl** solution into each test tube and mix it thoroughly by hand shaking.

5.4.4.6 Add **1ml** of **0.1M Barium Chloride** solution into each test tube. Mix it thoroughly by hand shaking.

5.4.4.7 Allow the tubes to stand at room temperature for **15 minutes**.

5.4.4.8 If the unknown sample is clear or only slightly turbid compared to the sulphate standard, the sample passes. To confirm the result, the absorbance of the sulphate standard and the unknown sample can be measured by the spectrophotometer against the Blank. (**Section 5.4.5**)

5.4.4.9 Record the result in the **DS** Notebook.

5.4.5 Measuring Absorbance of Standard and Unknown Powder Samples with Spectrophotometer

5.4.5.1 Refer to the SOPs# ***QC3112***

Operation and Maintenance Procedures - UV- 2101PC Spectrophotometer, Shimadzu

With Software **UV Probe** Version **1.11**

5.4.5.2 From top "**Window**" menu, Select "**2 Photometric**" mode.

5.4.5.3 From top "**Edit**" menu, Select "**Method**" to open "**Photometric Method**" sub-window.

5.4.5.3.1 Under the **Wavelength** tab, set up as:

Select - **Wavelength type: Point**
Enter - **Wavelength (λ, nm): 420.0nm**
Select - **Data Acquired by: ◘ Instrument**

5.4.5.3.2 In the **"Entries"** square, click the **"Add"** button to add "Wavelength type: **Point** and Wavelength (λ, nm) **420.0nm"** to finish setting.

5.4.5.4 Click on the "**Close**" button to close the sub-window.

5.4.5.5 Consequently, the **Sample Table** shows **[Active]** on the screen.

5.4.5.6 Fill two cuvettes with the labelled **Blank** solution (prepared in step **5.4.4**) as Reference Solution. Place one in the "Background Beam" compartment, and one in the "Sample Beam" compartment.

5.4.5.7 Click on the **"Auto Zero"** button to zero the double beams of the spectrophotometer.

5.4.5.8 Under **Sample ID**, enter a batch number in the cell and press the "**Enter**" key.

5.4.5.9 Take out the front "**Sample Beam**" cuvette and discard the Blank.

5.4.5.10 Fill the cuvette with the sulphate standard solution (prepared in step **5.4.4**) and rinse it a few times.

5.4.5.11 Re-fill the sulphate standard solution into the cuvette and place it into the front "**Sample Beam**" compartment.

5.4.5.12 Click on the "**Read Unknown**" button to obtain the absorbance against the Blank.

5.4.5.13 Record the absorbance results in the **DS** notebook.

5.4.5.14 Take out the front "**Sample Beam**" cuvette and discard the sulphate standard solution.

5.4.5.15 Repeat steps from **5.4.5.11** to **5.4.5.14** to obtain a duplicate result.

5.4.5.16 Fill the cuvettes with the unknown sample solution (prepared in step **5.4.4**) and rinse it a few times.

5.4.5.17 Re-fill the unknown sample solution into the cuvette and place it into the front "**Sample Beam**" compartment.

5.4.5.18 Click on the "**Read Unknown**" button to obtain the absorbance against the Blank.

5.4.5.19 Record the absorbance results in the **DS** notebook.

5.4.5.20 Take out the front "**Sample Beam**" cuvette and discard the unknown sample solution.

5.4.5.21 Repeat steps from **5.4.5.17** to **5.4.5.20** to obtain a duplicate result.

5.4.5.22 Remove both cuvettes and clean with distilled water and leave to dry.

5.4.6. Expected Results

5.4.6.1 A colloidal form of barium sulphate precipitate can be observed in the presence of Free Inorganic Sulphate in the solution. The standard sulphate solution (0.2 % SO_4^{-2}) would show very high turbidity within 1-2 minutes. However, the unknown sample solution would remain clear for 15 minutes.

5.4.6.2 If the absorbance of the unknown sample solution is lower than that of the standard sulphate solution (0.2 % SO_4^{-2}) or the same as that of the standard sulphate solution (0.2 % SO_4^{-2}), it passes.

5.4.6.3 If the absorbance of the unknown sample solution is higher than that of the standard sulphate solution (0.2 % SO_4^{-2}), it fails.

5.5 RESIDUE ON IGNITION TEST

Sample
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Desiccator with Silica Gel
Furnace
Fume Hood
A pair of Crucible Tongs
A pair of Heat Resistant Gloves
Crucible
Spatula
Weighing Boat/Paper

5.5.1 Preparing Unknown Powder Samples

- **The sample weight used for the result calculation is on a Dry Basis.**

5.5.1.1 Wearing a pair of Heat Resistant gloves and using a pair of crucible tongs, place two empty crucibles into the furnace and heat it up to **600°C**.

5.5.1.2 Turn the furnace off. Wearing a pair of Heat Resistant gloves and using a pair of crucible tongs, transfer the empty crucibles from the furnace into a desiccator to reach room temperature.

5.5.1.3 Using a pair of crucible tongs, take the empty crucibles out of the desiccator and weigh them.

5.5.1.4 Record the empty crucibles' weights in the **DS** notebook (**weight 1**).

5.5.1.5 Using a spatula, accurately weigh between **1.0000g to 1.5000g** of Dextran Sulphate powder sample in the pre-weighed empty crucible directly.

5.5.1.6 Record the total weights of the powder sample and the crucible before heating in the **DS** notebook (**weight 2**).

5.5.2 Igniting Unknown Powder Samples

5.5.2.1 Using a pair of crucible tongs, place the sample crucibles in the furnace and turn the power on.

5.5.2.2 Observe the temperature rise. When the temperature reaches about **810°C**, maintain for **10 minutes**.

5.5.2.3 When the time is up, turn the furnace off and wait for about **20 minutes**.

5.5.2.4 Wearing a pair of Heat Resistant gloves and using a pair of crucible tongs, take the sample crucibles out from the furnace.

5.5.2.5 Place the sample crucibles in the desiccator and allow them to reach room temperature.

5.5.2.6 Using a pair of crucible tongs, re-weigh the sample crucibles containing the residue after heating.

5.5.2.7 Record the weights in the **DS** notebook (**weight 3**).

5.5.2.8 Determine percentage (**w/w**) of the residue on ignition in Dextran Sulphate powder sample from the weight differences before ignition and after ignition.

5.5.3 Calculating Unknown Powder Samples

$$\textbf{Residue on Ignition \% (w/w)} = \frac{\textbf{(Weight 3 – Weight 1)}}{\textbf{(Weight 2 – Weight 1) – LOD}} \times 100\%$$

$$= \frac{\textbf{(Weight 3 – Weight 1)}}{\textbf{Net Sample Weight}} \times 100\%$$

Where:

- Weight 1 = Weight of empty crucible (g)
- Weight 2 = Weight of crucible & powder sample before ignition (g)
- Weight 3 = Weight of crucible & sample residue after ignition (g)
- LOD = Loss on Drying in %
- Net Sample Weight = Weight of unknown powder on a Dry Basis (g)
- 100% = Result unit conversion from g to % (w/w)

5.6 TOTAL SULPHUR CONTENT

Sample, Reagents and Solutions
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)
Concentrated Hydrochloric Acid (HCl, 36.5% ~38%)
Concentrated Nitric Acid (HNO_3)
Barium Chloride Dihydrate ($BaCl_2 \bullet 2H_2O$) Crystals
0.1 N Silver Nitrate ($AgNO_3$) Solution
Distilled Water

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated Analytical Balance, A and D EJ-610 or Equivalent (0.00)
Desiccator with Silica Gel
Micro-Pipette, 0.5 ~ 5-ml (adjustable) and Pipette Tips
Fume Hood
Furnace
Hot/Stirring Plate
Magnetic Stirring Bar
Sterile Nitrocellulose Filter Paper, Millipore, 0.45µm
Suction Filtration Apparatus:
- Vacuum Filtration Pump
- Graduated Filtration Funnel 300-ml
- Filter Holder - Fritted Glass Support Base & Silicone Stopper, No.8
- Graduated Erlenmeyer Filtering Flask 500ml
- Anodized Aluminum Clamp

Glassware and Accessories
Spatula
Weighing Paper/Boat
Beaker, 100-ml
Volumetric Flask with stopper, 100-ml
Watch Glass
Test Tubes
Glass Filtering Funnel
Whatman Ashless Filter Paper, 42
Pair of Heat Resistant Gloves
Pair of Crucible Tongs

Glassware and Accessories (cont'd)
Crucible
Unitary Wash Bottle
Pasteur Pipette and Rubber Bulb
Labels

5.6.1 Preparing Unknown Powder Sample Solution

- **Prepare each sample in duplicate.**
- **The sample weight used for the result calculation is on a Dry Basis.**

5.6.1.1 Using a spatula, accurately weigh between **1.0000g to 1.2000g** of Dextran Sulphate powder sample in a weighing boat. Then transfer it to a 100-ml beaker.

5.6.1.2 Record the powder sample weight in the **DS** notebook (**weight 1**).

5.6.1.3 Rinse the weighing boat with about **5 to 10ml** of distilled water and add the rinsings into the beaker.

5.6.1.4 Pipette **10ml** of **concentrated HCl,** 36.5% ~38%, solution into the 100-ml beaker.

5.6.1.5 Pipette **3ml** of **concentrated HNO_3** solution into the 100-ml beaker.

5.6.1.6 After rinsing and adding, a total volume of around **30ml** mixed solution is obtained. Then cover the beaker with a watch glass.

5.6.1.7 Place the beaker on the hot/stirring plate. Mix the solution with a magnetic stirring bar.

5.6.1.8 Boil the solution for **30 minutes**. During boiling, keep adding distilled water to maintain the volume **above 20ml**.

5.6.1.9 Using a spatula, accurately weigh about **2.00g** of $BaCl_2 \cdot 2H_2O$ crystals in a weighing boat.

5.6.1.10 Record the crystals weight in the **DS** notebook.

5.6.1.11 Cautiously transfer the **$BaCl_2$** crystals into the boiling beaker. Then continue boiling the final resulting solution further **10 minutes**.

5.6.1.12 Add distilled water into the beaker to make the volume up to about **80ml**. Then cool the solution down to room temperature.

5.6.2 Filtration of Sediment and Washing out Chloride from Unknown Powder Sample

5.6.2.1 Prepare and place two crucibles in the furnace. Turn the furnace power on and heat the crucibles up to **600°C**.

5.6.2.2 Turn the furnace power off. Using a pair of heat resistant gloves and crucible tongs, take out the crucibles from the furnace and place them in a desiccator immediately to reach room temperature ready for use.

5.6.2.3 Label a **0.45µm** Sterile Nitrocellulose Filter Paper with Batch No., Run No., and Date.

5.6.2.4 Prepare the filtration apparatus:
- insert the stopper surrounded filter holder into a 500-ml erlenmeyer filtration flask.
- place the labelled filter paper on the fritted glass support base of the filter holder.
- place a graduated filtration funnel to cover the filter paper.
- clamp both filter holder and graduated funnel together with an aluminum clamp.

5.6.2.5 Connect the filtration apparatus to the vacuum pump.

5.6.2.6 Turn the pump on and observe the vacuum reach about **-25Hg**. Wait until the vacuum is stable.

5.6.2.7 Swirl the beaker to mix the sample solution and sediment and pour into the filtration funnel. Rinse the beaker with Distilled water to ensure all sediment is transferred to the filtration funnel.

5.6.2.8 Transfer **10ml** of the filtrate into a test tube. Discard the rest and wash the erlenmeyer flask carefully rinsing with Distilled Water.

5.6.2.9 Pipette **1ml** of **concentrated HNO_3** solution into the test tube. Mix well by hand shaking.

5.6.2.10 Pipette **1 ml** of **0.1N $AgNO_3$** solution into the test tube as well and observe if the sediment still forms.

- If there is sediment of **Silver Chloride (AgCl)** formed, it indicates that the chloride is still present in the powder sample and has to be washed out.

5.6.2.11 Continue adding distilled water into the graduated filtration funnel to wash out the chloride in the unknown powder sample.

5.6.2.12 Repeatedly perform steps ***5.6.2.8*** to ***5.6.2.11*** to filter away chloride in the sample through the **0.45µm** filter paper until there is no **AgCl sediment** in the filtrate.

5.6.2.13 When the filtration is completed, unplug the vacuum, release the aluminum clamp, and then remove the **0.45µm** filter paper.

5.6.2.14 Place a piece of Whatman ashless filter paper inside a pre-cleaned glass filtering funnel.

5.6.2.15 Using a unitary wash bottle with distilled water, rinse and carefully transfer the sediment, which is **Barium Sulphate ($BaSO_4$),** from the **0.45µm** filter paper to the Whatman ashless filter paper.

5.6.3 Igniting Unknown Powder Samples

5.6.3.1 Using a pair of crucible tongs, take out the preheated crucibles from the desiccator and weigh the empty crucibles (**weight 2**).

5.6.3.2 Record the weights in the **DS** notebook.

5.6.3.3 Place the Whatman ashless filter paper containing the **$BaSO_4$** sediment into the pre-heated and pre-weighted empty crucibles.

5.6.3.4 Using a pair of crucible tongs, place the crucibles containing the Whatman ashless filter paper into the furnace and turn the power on.

5.6.3.5 Observe the temperature increasing. When the temperature reaches about **810°C**, maintain it for **20 minutes**.

5.6.3.6 When the time is up, turn the furnace off and wait for about **20 minutes**.

5.6.3.7 Wearing a pair of Heat Resistant gloves and using a pair of crucible tongs, take the sample crucibles out from the furnace.

5.6.3.8 Place the sample crucibles in the desiccators immediately. Then allow them to reach room temperature (**about 20 minutes**).

5.6.3.9 Using a pair of crucible tongs, take out the sample crucibles from the desiccator, re-weigh the crucibles after heating to get the residue weight (**weight 3**).

5.6.3.10 Record the residue weight in the **DS** notebook.

5.6.3.11 Determine percentage (**w/w**) of Total Sulphur Content in DS40 powder sample from the weight difference before ignition and after ignition in step **5.6.4.**

5.6.4 Calculating Total Sulphur Content in Unknown Powder Samples

$$\text{\% Total Sulphur Content (w/w)} = \frac{(\text{Weight 3} - \text{Weight 2}) \times 0.1374}{\text{Weight 1 - LOD}} \times 100\%$$

$$= \frac{(\text{Weight 3} - \text{Weight 2}) \times 0.1374}{\text{Net Sample Weight}} \times 100\%$$

Where:

- Weight 1 = Weight of Dextran Sulphate powder sample before heating (g)
- Weight 2 = Weight of empty crucible after heating (g)
- Weight 3 = Weight of crucible & $BaSO_4$ sediment after heating (g)
- 0.1374 = $\frac{\text{Molecular Weight of S (32.066)}}{\text{Molecular Weight of } \mathbf{BaSO_4} \text{ (233.392)}}$
- LOD = Loss on Drying in %
- Net Sample Weight = Weight of unknown powder on a Dry Basis (g)
- 100% = Result unit conversion from g to % (w/w)

5.7 GLUCOSE CONTENT (Modified BP Method)

Sample and Standard
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)
Dextrose (D-Glucose, Anhydrous, ACS specifications)

Reagents and Solutions
Concentrated Sulphuric Acid (H_2SO_4, 95% ~ 98%), ACS Grade
Anthrone Powder
Distilled Water
Reverse Osmosis Water (RO Water)

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated Spectrophotometer, UV-2101PC, Shimadzu, or Equivalent
Spectrophotometer Cuvettes
Stirring Plate
Magnetic Stirring Bar
Fume Hood
Boiling Cooker
Gas Burner and Lighter
A Pair of Cotton Gloves
Freezer
Fridge
Micro Pipette, 0.5-ml ~ 5-ml (adjustable), 1-ml, and Tips
Timer

Glassware and Accessories
Spatula
Weighing Paper/Boat
Beaker, 100-ml
Volumetric Flasks with Stoppers, 100-ml, 500-ml, 1000-ml
Graduated Cylinder
Erlenmeyer Flask with Rubber Stopper, 250-ml or Equivalent
Test Tubes with Glass Stoppers, Borosilicate Glass, 19x150mm, Kimax
Pasteur Pipette and Rubber Bulb
Ice Container
Ice cubes and trays

Glassware and Accessories (cont.)
Bottle and Bottle-Top Dispenser
Aluminum Foil
Metal Rack
Unitary Wash Bottle
Utility Wipers
Labels

- **All glassware and glass test tubes must be dried prior to use.**
- **The Anthrone Solution is sensitive to oxygen. Prepare it fresh on the day of use.**

5.7.1 Preparing Anthrone Solution in 90% ~ 93% H_2SO_4

5.7.1.1 Pipette **10ml** of distilled water into a 250-ml erlenmeyer flask.

5.7.1.2 Measure **190ml** of Concentrated **Sulphuric Acid (H_2SO_4)** solution with a graduated cylinder and slowly add it into the erlenmeyer flask as well.

5.7.1.3 Mix the diluted acid solution well with a magnetic stirring bar on a stirring plate in the fume hood.

5.7.1.4 Using a spatula, accurately weigh about **400mg (0.4000g)** of **Anthrone** powder on a piece of weighing paper.

5.7.1.5 Record the reagent weight in the Lab Reagent notebook.

5.7.1.6 Quickly and cautiously transfer Anthrone powder into the erlenmeyer flask while the diluted **H_2SO_4** solution is mixing.

5.7.1.7 Rinse the weighing paper with a few drops of distilled water, add the rinsings into the erlenmeyer flask as well. Cover it with a rubber stopper and wrap the entire erlenmeyer flask with a piece of aluminum foil.

5.7.1.8 Continue mixing the resulting solution until Anthrone powder is completely dissolved and then cool down to room temperature.

- Complete the preparation within **30 minutes** due to oxygen sensitivity of Anthrone reagent.

5.7.1.9 Transfer Anthrone solution into a brown bottle with a bottle-top dispenser and place it in the freezer until the time of use.

5.7.2 Preparing D-Glucose Standard Stock Solution (G.S.S)

5.7.2.1 Using a spatula, accurately weigh about **440mg (0.4400g)** of **Dextrose(D-Glucose)** standard Powder on a piece of weighing paper. Then transfer to a 100-ml beaker.

5.7.2.2 Record the standard powder weight in the Lab Reagent notebook.

5.7.2.3 Rinse the weighing paper with distilled water and add the rinsings into the beaker.

5.7.2.4 Continue adding about **80ml** of distilled water into the 100-ml beaker as well.

5.7.2.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.7.2.6 Transfer the solution into a 1000-ml volumetric flask.

5.7.2.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask as well.

5.7.2.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

- Label the **G.S.S** solution with a known nominal concentration of about **440µg/ml** of pure **D-Glucose**.

5.7.3 Preparing Different Standard Point Solutions for a Calibration Curve

5.7.3.1 Pipette **3.0ml** of **G.S.S.** into a 100-ml volumetric flask (nominal concentration **13.2µg/ml)**.

5.7.3.2 Pipette **7.5ml** of **G.S.S.** into a 100-ml volumetric flask (nominal concentration **33.0µg/ml)**.

5.7.3.3 Pipette **12.0ml** of **G.S.S.** into a 100-ml volumetric flask (nominal concentration **52.8µg/ml)**.

5.7.3.4 Dilute each standard solution with distilled water up to volume. Mix them well by hand shaking. Let stand for a while to be evenly dissolved and to cool down after shaking.

5.7.3.5 From step **5.7.3.1** to **5.7.3.4,** each volumetric flask contains different point mixture of **G.S.S.** and distilled water with nominal concentrations in **µg/ml** as below:

G.S.S.(ml) Taken	Final Volume (ml)	Nominal Glucose (µg/ml)
3.0	100	13.2
7.5	100	33.0
12.0	100	52.8

5.7.4 Preparing Unknown Powder Sample Solutions

- **The sample weight used for the result calculation is on a Dry Basis.**

5.7.4.1 Using a spatula, accurately weigh between **1.0000g to 1.5000g** of **Dextran Sulphate** powder sample in a weighing boat. Then transfer it to a 100-ml beaker.

5.7.4.2 Record the powder sample weight in the **DS** notebook.

5.7.4.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.7.4.4 Continue adding about **60ml** of distilled water into the beaker as well.

5.7.4.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.7.4.6 Transfer the solution into a 200-ml volumetric flask.

5.7.4.7 Rinse the original beaker with distilled water and add the rinsings into the 200-ml volumetric flask as well.

5.7.4.8 Dilute the resulting solution with distilled water up to volume. Mix well on the stirring plate with a magnetic stirring bar.

5.7.4.9 Leave the prepared solution at room temperature until it cools down after mixing.

5.7.4.10 Pipette **10.0ml** of the resulting solution into a 1000-ml volumetric flask.

5.7.4.11 Dilute the resulting solution with distilled water up to volume. Mix well on the stirring plate with a magnetic stirring bar.

5.7.5 Preparing a Boiling Water Bath

5.7.5.1. Fill a cooker around 2/3 full with RO water and cover it with the lid.

- Ensure that the water level is sufficient to cover the **9ml** of sample mixture in the test tube.

5.7.5.2. Turn on the gas burner to boil the water.

- Ensure that the water is vigorously boiling (i.e.100°C) before placing the metal rack containing the sample tubes into the water bath.

5.7.6 Preparing Standards, Blank, & Unknown Samples with Anthrone Solution

- **Prepare each sample in duplicate and keep them in an ice-bath.**

5.7.6.1 Prepare an ice-water bath by placing two to three trays of ice cubes into an ice container and fill it with RO water to at least ¾ of the container.

5.7.6.2 Label all required glass test tubes and insert them into a metal rack.

5.7.6.3 Pipette **3.0ml** of distilled water into the labelled test tubes as **Blank**.

5.7.6.4 Pipette **3.0ml** of each standard point solution prepared in step **5.7.3** into the labelled test tubes, respectively.

5.7.6.5 Pipette **3.0ml** of the Lab Control sample solution into labelled test tubes.

5.7.6.6 Pipette **3.0ml** of the Unknown sample solution prepared in step **5.7.4.11** into labelled test tubes, respectively.

5.7.6.7 Place the metal rack containing the sample tubes into the ice-water bath.

5.7.6.8 Take out cold Anthrone solution equipped with a bottle-top dispenser from the freezer.

5.7.6.9 Carefully dispense **6.0ml** of the Anthrone solution into each sample test tube:

- Two distinct layers are observed. The Anthrone solution is the bottom layer.
- The mixing of the sample and Anthrone solution is extremely exothermic.

5.7.6.10 Place the stopper on the test tubes and immediately mix the contents well by carefully inverting the tubes **5-6** times. Then ensure that the stoppers are loosened.

5.7.6.11 Wearing a pair of cotton gloves, set aside the cooker lid.

5.7.6.12 Take the metal rack containing the sample tubes out from the ice-water bath, but keep the ice-water bath for use after boiling.

5.7.6.13 Place the metal sample rack into the boiling water bath immediately with caution. Cover it with a piece of aluminum foil.

5.7.6.14 Set **9 minutes** with a timer to ensure 5 minutes of boiling time.

5.7.6.15 When the time is up, wear a pair of cotton gloves and cautiously take the metal sample rack out of the boiling water bath.

5.7.6.16 Immediately place the metal sample rack back into the ice-water bath to stop reaction for **4-5 minutes**.

5.7.6.17 Take the metal sample rack out from the ice-water bath. Allow it to stand on the counter for about **30 minutes** until the tubes reach room temperature.

- Be cautious when placing and removing the sample rack from the boiling water bath.

5.7.6.18 By operating the spectrophotometer, generate a Standard Calibration Curve and then obtain Glucose Content for the Lab Control and the Unknown DS40 powder samples.

- During the spectrophotometer measurements, use Pasteur pipette to transfer a prepared sample solution from the test tube to the cuvettes

5.7.7 *Measuring Absorbance of Unknown Powder Sample with Spectrophotometer*

5.7.7.1 Refer to the SOP# ***QC3112***

Operation and Maintenance Procedures - UV- 2101PC Spectrophotometer, Shimadzu

With Software **UV Probe** Version **1.11**

5.7.7.2 From top "**Window**" menu, Select "**2 Photometric**" mode.

5.7.7.3 From top "**Edit**" menu, Select "**Method**" to open "**Photometric Method**" sub-window.

5.7.7.3.1 Under the **Wavelength** tab, set up as:

Select - **Wavelength type: Point**
Enter - **Wavelength (λ, nm): 625.0nm**
Select - **Data Acquired by: ◘ Instrument**

5.7.7.3.2 In the **"Entries"** square, click **"Add"** to add "Wavelength type: **Point** and Wavelength (λ, nm) **625.0nm"** to finish setting.

5.7.7.3.3 Under the "**Instrument Parameters**" tab, set up as:

Select - Measuring Mode "**Absorbance**"; **Y-axis**

5.7.7.3.4 Select "**Calibration**" tab, set up as:

Select - Calibration Type "Multi Point"
Select - Formula "Fixed Wavelength"
Enter - Concentration Units "µg/ml"; X-axis
Select - Parameters Con = f (Abs)
Select - Order of Curve 1st
Un-Tick - □ "Zero Interception"

5.7.7.4 Click on "**Close**" to close the sub-window.

5.7.7.5 Fill two cuvettes labelled **Blank** solution (step **5.7.6.17**) as Reference Solution. Place one in the "Background Beam" compartment and one in the "Sample Beam" compartment.

5.7.7.6 Click on **"Auto Zero"** to zero the double beams of the spectrophotometer.

5.7.7.7 On the "**Standard Table**":

5.7.7.7.1 Under the "**Sample ID**" cell, enter a number for the 1st standard point and press the "**Enter**" key.

5.7.7.7.2 Under the "**Concentration**" cell, enter the concentration in **µg/ml** of the 1st Standard point (**13.2µg/ml**) as shown in step **5.7.3.5** table.

5.7.7.7.3 Take out the front "**Sample Beam**" cuvette and discard the **Blank**.

5.7.7.7.4 Fill the cuvette with the 1st standard solution a few times to rinse it.

5.7.7.7.5 Re-fill the 1st standard solution into the cuvette and place it into the front "**Sample Beam**" compartment.

5.7.7.7.6 Click on "**Read Std**" to obtain an absorbance reading for the 1st standard point.

5.7.7.7.7 Repeat step **5.7.7.7.1** to **5.7.7.7.6** entering the 2nd Standard point (**33.0µg/ml**) and the 3rd Standard point (**52.8µg/ml**), respectively. Obtain absorbance readings for each concentration point to generate a standard calibration curve.

5.7.7.8 On the "**Sample Table**":

5.7.7.8.1 Under the "**Sample ID**" cell, enter the Lab Control batch number and press the "**Enter**" key.

5.7.7.8.2 Fill the cuvette with the Lab Control sample solution a few times to rinse it.

5.7.7.8.3 Re-fill the Lab Control sample solution into the cuvette and place it into the front "**Sample Beam**" compartment.

5.7.7.8.4 Click on "**Read Unknown**" to obtain a Glucose concentration in **µg/ml** for the Lab Control sample solution against the standard calibration curve.

5.7.7.8.5 Take out the front "**Sample Beam**" cuvette and discard the Lab Control sample.

5.7.7.8.6 Repeat step **5.7.7.8.1** to **5.7.7.8.5,** entering each individual batch number, to obtain all unknown powder sample Glucose concentrations in **µg/ml**, in sequence.

5.7.7.8.7 Remove both cuvettes and clean with distilled water and leave to dry.

5.7.7.8.8 Print out the data and graphs for the Calibration Curve and the unknown samples and perform calculations from step 5.7.8.

5.7.7.8.9 Record results in the **DS** notebook.

5.7.8 Calculation of Standard Calibration Curve and Unknown Powder Sample

5.7.8.1 Formula for the preparation of Standard Calibration Curve

$$\textbf{D-Glucose content (µg/ml)} = \frac{\textbf{440 x G.S.S Volume}}{\textbf{100}}$$

Where:

- 440 = Nominal Concentration of D-Glucose in G.S.S.S (µg/ml)
- G.S.S. Volume = Volume in ml taken for calibration curve preparation in Step.5.7.3.
- 100 = Final volume of standard calibration curve preparation in Step 5.7.3.

5.7.8.2 Formula for Calculation of Unknown Powder Sample

$$\textbf{Glucose Content \%(w/w)} = \frac{C_{SMP} \textbf{ x 200 x 1000/10}}{\textbf{(Weight of DS40 Powder -LOD) x 1,000,000}} \textbf{ x 100\%}$$

$$= \frac{C_{SMP} \textbf{ x 200 x 100}}{\textbf{(Weight of DS40 Powder – LOD) x 1,000,000}} \textbf{ x 100\%}$$

$$= \frac{C_{SMP}}{\textbf{Net Sample Weight x 50}} \textbf{ x 100\%}$$

Where:

- C_{SMP} = Spectrophotometer reading of D-Glucose Content in unknown powder sample solution (µg/ml)
- 10 = Volume taken during Sample Preparation in ml
- 200, 1000 = Dilution Volumes for Sample Preparation in ml
- Weight of DS40 Powder = Weight of Unknown Powder Sample (g)
- LOD = Loss on Drying in %
- 1,000,000 = Conversion from g/ml to µg/ml
- Net Sample Weight = Weight of unknown powder sample on a Dry Basis (g)
- 100% = Unit Conversion of the Result from µg/ml to % (w/w)

5.8 SPECIFIC VISCOSITY

Sample, Reagents and Solutions
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)
Sodium Chloride (NaCl) Powder
Distilled Water
Reverse Osmosis Water (RO Water)

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated Analytical Balance, A and D EJ-610 or Equivalent (0.00)
Viscometer Ubbelohde # 1 (Refer to Figure 1.)
Viscometer Holder
Immersion Circulator in a Water Bath Set **25 ± 0.1° C**
Pipette Filler
Timer
Stirring Plate
Magnetic Stirring Bar

Glassware and Accessories
Spatula
Weighing Paper/Boat
Beaker, 100-ml
Volumetric Flask with stopper, 100-ml, 1000-ml
Unitary Wash Bottle
Labels

5.8.1 Preparing 1.0M Sodium Chloride (NaCl) Solution (Blank)

5.8.1.1 Using a spatula, accurately weigh about **58.44g** of Sodium Chloride powder in a weighing boat. Then transfer it to a 100-ml beaker.

5.8.1.2 Record the reagent powder weight in the Lab Reagent notebook.

5.8.1.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.8.1.4 Continue adding about 50ml of distilled water into the 100-ml beaker as well.

5.8.1.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.8.1.6 Transfer the solution into a 1000-ml volumetric flask.

5.8.1.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask as well.

5.8.1.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.8.1.9 Transfer the final resulting solution into a storage bottle and label it.

5.8.2 Preparing 1% Unknown Powder Sample (1%D.S) in 1.0M NaCl Solution

- **The sample weight used for the result calculation is on a Dry Basis.**

5.8.2.1 Using a spatula, accurately weigh about **1.0000g to 1.2000g** of **Dextran Sulphate** powder sample on a piece of weighing paper. Then transfer it to a 100-ml beaker.

5.8.2.2 Record the powder sample weight in the **DS** notebook.

5.8.2.3 Rinse the weighing paper with **1.0M NaCl** solution, prepared in step **5.8.1**, and add the rinsings into the beaker.

5.8.2.4 Continue adding about **50ml** of **1.0M NaCl** solution into the beaker as well.

5.8.2.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder sample is completely dissolved.

5.8.2.6 Transfer the sample solution into a 100-ml volumetric flask.

5.8.2.7 Rinse the original beaker with **1.0M NaCl** solution and add the rinsings into the volumetric flask as well.

5.8.2.8 Dilute the resulting solution with **1.0M NaCl** solution up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.8.3 Measuring Efflux Time of Blank

5.8.3.1. Prepare a clean Ubbelohde Viscometer and a water bath filled with RO water at **25°C**

5.8.3.2. Fill the viscometer with **1.0M NaCl** solution, prepared in step **5.8.1**, through tube **C** into the main reservoir between the marks, **H** and **I**.

5.8.3.3 Cover tube **B** with a finger. Using a pipette filler, suck **1.0M NaCl** solution from the top of tube **A** to bring the solution level up to the middle of the small bulb **D**.

5.8.3.4 Remove the pipette filler and finger to allow **1.0M NaCl** solution to flow freely down to rinse the walls of the viscometer.

5.8.3.5 Discard **1.0M NaCl** solution after rinsing the viscometer.

5.8.3.6 Fill the viscometer with **1.0M NaCl** solution through tube **C** into the main reservoir between the marks, **H** and **I**, again.

5.8.3.7 Place the viscometer into the holder and then into the water bath at **25°C**.

5.8.3.8 Allow the viscometer to stand for approximately **5 minutes** to equilibrate to the water bath temperature.

5.8.3.9 Cover tube **B** with a finger. Using a pipette filler, suck **1.0M NaCl** solution from the top of tube **A** to bring the solution level up to the middle of the small bulb **D**.

5.8.3.10 Remove the pipette filler and finger to allow **1.0M NaCl** solution to flow freely down. In the meanwhile, start the timer to measure the efflux time for the meniscus of the liquid passing from the top mark **F** to the bottom mark **G**.

5.8.3.11 Record the efflux time in seconds.

5.8.3.12 Repeat steps from **5.8.3.9** to **5.8.3.11** until a stable reading is established.

5.8.3.13 Take the viscometer out of the water bath and discard **1.0M NaCl** solution.

5.8.4 Measuring Efflux Time of Unknown Powder Samples

5.8.4.1 Fill the viscometer with **1% D.S** sample solution prepared in step **5.8.2**, through tube C into the main reservoir between the marks, **H** and **I**.

5.8.4.2 Cover tube **B** with a finger. Using a pipette filler, suck **1% D.S** sample solution from the top of tube **A** to bring the solution level up to the middle of the small bulb **D**.

5.8.4.3 Remove the pipette filler and finger to allow **1% D.S** sample solution to flow freely down to rinse the walls of the viscometer.

5.8.4.4 Discard **1% D.S** sample solution after rinsing the viscometer.

5.8.4.5 Fill the viscometer with **1% D.S** sample solution through tube **C** into the main reservoir between the marks, **H** and **I,** again.

5.8.4.6 Place the viscometer into the holder and then into the water bath at **25°C**.

5.8.4.7 Allow the viscometer to stand for approximately **5 minutes** to equilibrate to the water bath temperature.

5.8.4.8 Cover tube **B** with a finger. Using a pipette filler, suck **1% D.S** sample solution from the top of tube **A** to bring the solution level up to the middle of the small bulb **D**.

5.8.4.9 Remove the pipette filler and finger to allow **1% D.S** sample solution to flow freely down. In the meantime, start the timer to measure the efflux time for the meniscus of the liquid passing from the top mark **F** to the bottom mark **G**.

5.8.4.10 Record the efflux time in seconds.

5.8.4.11 Repeat steps from **5.8.4.8 to 5.8.4.10** until a stable reading is established.

5.8.4.12 Take the viscometer out of the water bath and discard the **1% D.S** sample solution.

5.8.4.13 Wash and rinse the viscometer with RO water/distilled water a few times.

5.8.4.14 Then fill the viscometer with RO water/distilled water through tube **C**, up to mark **D** of tube **A.** Then leave it in the water bath for next use.

5.8.4.15 Calculate the Specific Viscosity following the formula in step **5.8.5** and record the result in the **DS** notebook.

5.8.5 Calculating Specific Viscosity of Unknown Powder Samples

$$\textbf{Specific Viscosity} = \frac{[(\text{ET of Sample Solution} / \text{ET}_0 \text{ of Blank}) - 1]}{\text{Weight of DS40 Powder - LOD}} - \frac{[(\text{ET of Sample Solution} / \text{ET}_0 \text{ of Blank}) - 1]}{\text{Net Sample Wt.}}$$

Where:

- ET = Efflux Time of sample solution in second
- Sample Solution = 1% DS40 unknown powder sample in 1.0M NaCl solution
- ET_0 = Efflux Time of Blank in second
- Blank = 1.0 M NaCl solution
- -1 = Eliminate Efflux Time of Blank from that of powder sample
- Weight of DS40 Powder = Weight of unknown powder sample (g)
- LOD = Loss on Drying in %
- Net Sample Wt. = Weight of unknown powder sample on a Dry Basis (g)

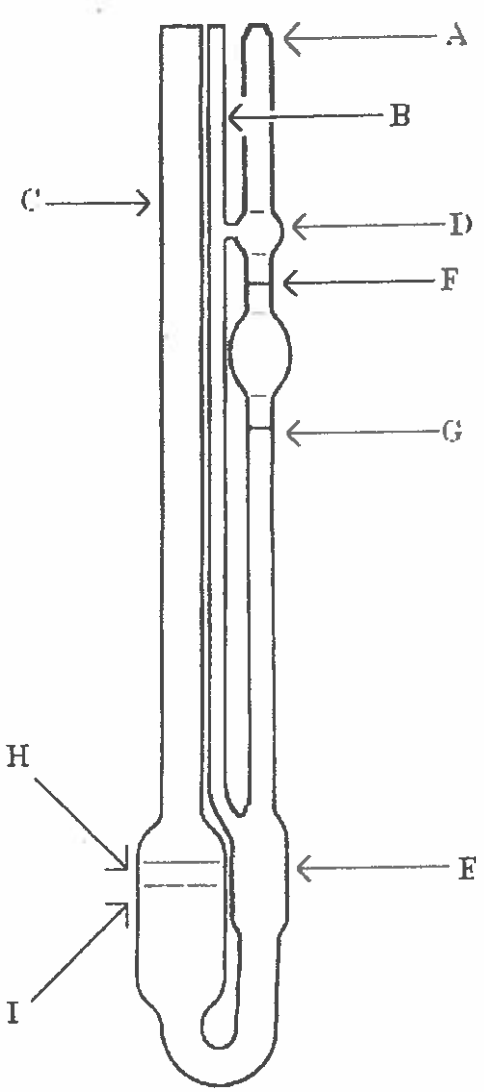

Figure 1. Ubbelohde Viscometer

Written By:		Title: QC Manager	Date: July 13, 2021
Reviewed By:		Title: Analytical Chemist	Date: July 13, 2021
Approved By:		Title: QA Manager	Date: July 13, 2021

5.9 pH Test

Sample and Reagents
1% Dextran Sulphate Sample Stock Solution (**1% D.S.S** from step ***5.3.11***)
Reference Standard pH 4.0 buffer, 500ml
Potassium Chloride in Reference Standard pH 4.0 buffer
Distilled Water
Reverse Osmosis Water (RO Water)

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated pH Meter ORION STAR A111 or equivalent
pH Electrode
Stirring Plate
Magnetic Stirring Bar

Glassware and Accessories
Spatula
Weighing Paper/Boat
Beaker, 100-ml, 600-ml
Unitary Wash Bottle
Utility Wipers
Labels

5.9.1 Preparing pH Electrode Storage Solution (2% Potassium Chloride in pH 4.0 buffer)

5.9.1.1 Using a spatula, accurately weigh about **10.00g** of potassium chloride powder in a weighing boat. Then transfer it to a 600-ml beaker.

5.9.1.2 Record the reagent weight in the Lab Reagent notebook.

5.9.1.3 Rinse the weighing boat with **pH 4.0 buffer**. Add the rinsings into the 600-ml beaker.

5.9.1.4 Continue adding the rest of the **500ml pH 4.0 buffer** into the beaker as well.

5.9.1.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the reagent powder is completely dissolved.

5.9.1.6 Transfer the resulting solution back to **pH 4.0 buffer** bottle. Label and store it at room temperature in dark.

5.9.2 Measuring pH of Unknown Powder Samples: Refer to SOP QC3105 for complete details on pH meter use

5.9.2.1 Obtain **1% D.S.S** solution, prepared in step **5.3.11**, and transfer at least **40ml** into a 100-ml beaker.

5.9.2.2 Press the "**Power**" button to turn the pH meter on.

5.9.2.3 Rinse the pH electrode with RO water/distilled water.

5.9.2.4 Place the pH electrode into the sample solution. Swirl or mix it well with the electrode.

- The solution must be sufficient to cover the white ring on the pH electrode (about **2.5cm**).

5.9.2.5 Press the "**Measure**" button to get a pH reading.

5.9.2.6 When the reading is complete, record the pH reading in the **DS** notebook.

5.9.2.7 Repeat **step 5.9.2.5 to 5.9.2.6** to obtain duplicate readings.

5.9.2.8 Take out the pH electrode from the sample solution. Rinse it completely with RO water/distilled water.

5.9.2.9 Wipe the pH electrode dry and place it into the **Storage Solution: 2% Potassium Chloride in Reference Standard pH 4.0 buffer.**

5.9.2.10 Press the "**Power**" button to turn the pH meter off.

5.10 SPECIFIC ROTATION

Sample and Reagents
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)
Distilled Water

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated Automatic Polarimeter, Digital, ADP440+ or Equivalent
Polarimeter Tube, Standard Glass, Length 1.0-dm /2.0-dm (100mm/200mm)
Stirring Plate
Magnetic Stirring Bar

Glassware and Accessories
Spatula
Weighing Boat
Beaker, 100-ml
Volumetric Flask with stopper, 100-ml
Unitary Wash Bottle
Utility Wipers
Labels

5.10.1 Preparing 5 % Dextran Sulphate Sample Solution (5% D.S)

- **The sample weight used for the result calculation is on a Dry Basis.**

5.10.1.1 Using a spatula, accurately weigh about **5.0000g** of **Dextran Sulphate** powder sample in a weighing boat. Then transfer it to a 100-ml beaker.

5.10.1.2 Record the powder sample weight in the **DS** notebook.

5.10.1.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.10.1.4 Continue adding about 50ml of distilled water into the 100-ml beaker as well.

5.10.1.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.10.1.6 Transfer the solution into a 100-ml volumetric flask.

5.10.1.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask as well.

5.10.1.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.10.1.9 Label the final resulting solution as **5% D.S**.

5.10.2 Measuring Optical Rotation of Unknown Powder Samples **(See SOP QC3109 for full details)**

➢ **Keep the sample chamber empty while the instrument is initializing or not in use.**

5.10.2.1 Press the "**power**" key to turn the Polarimeter on.

5.10.2.2 From the screen, confirm that all parameters are displaying correctly as set up:

- **Method:** Normal
- **Temperature:** General Chamber Temperature
- **Scale:** °a (angular)
- **Temperature Compensation:** Quartz
- **Range:** - 89° to +89°
- **Optical Density (O.D):** 0.0 (Max. 3.0)
- **Reading Response** Medium (20 Seconds)
- **Resolution** Medium (0.01)

5.10.2.3 After stabilizing, the polarimeter automatically resets to **zero** with **0.00** displayed on the screen. If it does not, press the "**Zero**" key.

➢ **Allow the polarimeter a further 30 minutes stabilising to take accurate readings.**

5.10.2.4 Obtain a pre-cleaned Polarimeter standard glass tube, **1.0-dm** usually or 2.0-dm.

5.10.2.5 Obtain **5% D.S sample solution** prepared in step **5.10.1.**

5.10.2.6 Rinse the polarimeter tube with **5% D.S sample solution** two to three times.

5.10.2.7 Fill **5% D.S sample solution** into the tube fully without creating any air bubbles.

5.10.2.8 Slide the clean glass cover disc from the side of the tube across the opening. Then cautiously place the cap, with the gasket (ring) inside, on top of the glass cover disc to close the end of the tube. It should be sealed securely, but not too tight.

5.10.2.9 Thoroughly wipe the tube dry, entire body and two ends, with absorbent paper towel.

5.10.2.10 Open the lower lid of the polarimeter. Place the tube on the stainless-steel rails inside the sample chamber and close it.

- **To obtain reliable data, never place a wet polarimeter tube in the sample chamber.**

5.10.2.11 A result is obtained when the angular reading for optical rotation changes from dark blue to black and is stable.

5.10.2.12 Record the measurement result in the **DS** notebook.

5.10.2.13 Open the lower lid to take out the tube from the sample chamber and discard the measured **5% D.S sample solution**.

5.10.2.14 Repeat steps **5.10.2.7** to **5.10.2.13** with **5% D.S sample solution** to obtain duplicate readings.

5.10.2.15 Wash and rinse the polarimeter tube with RO water/distilled water a few times.

5.10.2.16 After cleaning, fill the tube with RO water/distilled water to be ready for use.

5.10.2.17 To switch the Polarimeter off, press the "**power**" key and hold down for approximately 2 seconds.

5.10.2.18 Press the **"Yes"** button to enter **"Standby"**.

5.10.3 Calculating Specific Rotation of Unknown Powder Samples

Specific Rotation $[\alpha]_D^{20}$

$$= \frac{(°a \text{ Reading } 1 + °a \text{ Reading } 2)}{2(M)} \times \frac{\text{Volume of Sample}}{(\text{Wt. of DS40 Powder} - \text{LOD}) \times \text{Tube Length}}$$

$$= \frac{(O.R.\ 1 + O.R.\ 2)}{2(M)} \times \frac{100}{\text{Net Sample Weight} \times 1.0\text{-dm}},$$

Where:

- °a = Angular reading for Optical Rotation from the polarimeter
- 2(M) = To obtain a Mean value from duplicate readings
- Volume of Sample = Volume of unknown powder sample preparation, 100 ml
- Wt. of DS40 Powder = Weight of unknown powder sample (g)
- LOD = Loss on Drying in %
- Tube Length =1.0-dm Polarimeter Tube
  - If a 2.0-dm tube used, divide the reading by 2.
- O.R. = Optical Rotation
- Net Sample Wt.= Weight of unknown powder sample on a Dry Basis (g)

5.11 INSOLUBLE IRON

Sample and Standard
1% Dextran Sulphate Sample Stock Solution (**1% D.S.S** from step ***5.3.11***)
Iron (II) sulphate heptahydrate (Ferrous sulphate heptahydrate, $FeSO_4 \bullet 7H_2O$)

Reagent and Solutions
Hydroxylamine Hydrochloride ($NH_2OH \bullet HCl$)
Batho-phenanthroline [$(C_6H_5)_2C_{12}H_6N_2$] (4,7- Diphenyl-1,10-phenanthroline)
Sodium Acetate ($CH_3COONa \cdot 3H_2O$)
Chloroform ($CHCl_3$), Reagent Grade ACS, Assay 99.8%
Isopropyl Alcohol (I.P.A. $CH_3CHOHCH_3$), Reagent Grade ACS, Assay Min.99.5%
Distilled Water

Equipment and Accessories
Calibrated Analytical Balance, A&D, EJ610 or Equivalent (0.00)
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated Spectrophotometer, UV-2101PC, Shimadzu, or Equivalent
Spectrophotometer Cuvette
Fume Hood
Stirring Plate
Magnetic Stirring Bar
Micro-Pipette, 1-ml, 0.5ml~5.0ml (adjustable)
Micro-Pipette Tips

Glassware and Accessories
Spatula
Weighing Paper/Boat
Beaker, 250-ml/500-ml
Volumetric Flask with stopper, 100-ml, 500-ml, 1000-ml
Separatory Funnel with stopper, 60-ml
Separatory Funnel Rack
Bottle and Bottle-Top Dispenser
Pasteur Pipette and Rubber Bulb
Unitary Wash Bottle
Utility Wipers
Labels

5.11.1 Preparing 20% Hydroxylamine Hydrochloride Solution

5.11.1.1 Using a spatula, accurately weigh about **100.00g** of Hydroxylamine Hydrochloride powder in a weighing boat. Then transfer it into a 500-ml beaker.

5.11.1.2 Record the reagent powder weight in the Lab Reagent notebook.

5.11.1.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.11.1.4 Continue adding about 200ml of distilled water into the beaker as well.

5.11.1.5 Place the beaker on a stirring plate. Mix it with a magnetic stirring bar until the powder is completely dissolved.

5.11.1.6 Transfer the solution into a 500-ml volumetric flask.

5.11.1.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask.

5.11.1.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with the magnetic stirring bar.

5.11.1.9 Transfer the final resulting solution into a dark storage bottle equipped with a bottle-top dispenser and label it.

5.11.2 Preparing Batho-phenanthroline Solution

5.11.2.1 Using a spatula, accurately weigh about **0.3320g** of Batho-phenanthroline powder on a piece of weighing paper. Then transfer it into a 500-ml beaker.

5.11.2.2 Record the reagent weight in the Lab Reagent notebook.

5.11.2.3 Rinse the weighing paper with **I.P.A.(Min.99.5%)** and add the rinsings into the beaker.

5.11.2.4 Continue adding about 450ml of **I.P.A.** into the beaker as well.

5.11.2.5 Place the beaker on a stirring plate. Mix it with a magnetic stirring bar for at **least 2 hours** until the powder is completely dissolved.

5.11.2.6 Transfer the solution into a 1000-ml volumetric flask.

5.11.2.7 Rinse the 500-ml beaker with **I.P.A**. and add the rinsings into the volumetric flask as well.

5.11.2.8 Dilute the resulting solution with **I.P.A**. up to volume. Mix it well on the stirring plate with the magnetic stirring bar.

5.11.2.9 Transfer the final resulting solution into a dark storage bottle equipped with a bottle-top dispenser and label it.

5.11.3 Preparing 10% Sodium Acetate Solution

5.11.3.1 Using a spatula, accurately weigh about **100.00g** of Sodium Acetate powder in a weighing boat. Then transfer it into a 500-ml beaker.

5.11.3.2 Record the reagent weight in the Lab Reagent notebook.

5.11.3.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.11.3.4 Continue adding about 200ml of distilled water into the beaker as well.

5.11.3.5 Place the beaker on the stirring plate. Mix it with a magnetic stirring bar until the powder is completely dissolved.

5.11.3.6 Transfer the solution into a 1000-ml volumetric flask.

5.11.3.7 Rinse the original beaker with distilled water and add the rinsings into the volumetric flask as well.

5.11.3.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with the magnetic stirring bar.

5.11.3.9 Transfer final resulting solution into a dark storage bottle equipped with a bottle-top dispenser and label it.

5.11.4 Preparing Standard Solutions

5.11.4.1 *The Standard Stock* (S.S.) *Solution*

5.11.4.1.1 Prepare two clean and dry 100-ml volumetric flasks. Fill about 80ml of distilled water into one of the volumetric flasks.

5.11.4.1.2 Using a spatula, accurately weigh about **0.0500g (50.0mg)** of Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) standard powder, equivalent to**10mg** of pure $\mathbf{Fe^{2+}}$, on a piece of weighing paper. Then transfer it into the 100-ml volumetric flask containing about 80ml of distilled water.

5.11.4.1.3 Record the standard weight in the Lab Reagent notebook.

5.11.4.1.4 Rinse the weighing paper with distilled water. Add the rinsings into the volumetric flask.

5.11.4.1.5 Leave the standard solution at room temperature for a while until the powder is completely dissolved.

5.11.4.1.6 Dilute the resulting standard solution with distilled water up to volume. Mix it well by gentle hand shaking.

5.11.4.1.7 Leave the standard solution at room temperature for at **least 30 minutes** to cool down after shaking and before transferring.

5.11.4.2 *The Working Standard* (W.S.) *Solution*

5.11.4.2.1 Pipette **10.0ml** of the standard stock solution into another 100-ml volumetric flask and dilute it with distilled water up to volume.

5.11.4.2.2 Mix the working standard solution well by gentle hand shaking. Then allow it to stand at room temperature for at least another **30 minutes** before using.

5.11.4.2.3 A working standard with a known nominal concentration about **10µg$\mathbf{Fe^{2+}}$/ml** is obtained.

- An option is to weigh and dissolve **0.0500g (50.00mg)** of $FeSO_4 \cdot 7H_2O$ in a 1000-ml volumetric flask directly to obtain the same concentration as in step ***5.11.4.2.3***.

- $FeSO_4$ solution is oxidized slowly by air when cold, and rapidly when hot. The rate of oxidation is increased by exposure to light. Therefore, the standard stock solution is made in a volumetric flask directly with small amount of Standard for a very low concentration.

5.11.5 Preparing Pre-Extraction Solutions for Blank and Calibration Curve Points

- ***A new calibration curve should be prepared every time when new reagents are made.***

5.11.5.1 Label separatory funnels for Blank and different standard concentration points.

5.11.5.2 Pipette **10ml** of distilled water into a separatory funnel as **Blank**.

5.11.5.3 Pipette **1ml** of **W.S** solution and **9ml** of distilled water into a separatory funnel (nominal concentration **1.0µg/ml**).

5.11.5.4 Pipette **4ml** of **W.S** solution and **6ml** of distilled water into a separatory funnel (nominal concentration **4.0µg/ml**).

5.11.5.5 Pipette **10ml** of **W.S** solution into a separatory funnel (nominal concentration **10.0µg/ml**).

5.11.5.6 From step ***5.11.5.2*** to ***5.11.5.5***, each separatory funnel contains distilled water or working standard solutions with three different nominal concentration points in **µg/ml** and equivalent value **in %** summarized below.

W.S. Volume (ml)	Distilled Water (ml)	Total Volume (ml)	Nominal [ ] (µg/ml)	Equivalent [ ] (%)
0	10	10	0.0	0.0% (Blank)
1	9	10	1.0	0.05%
4	6	10	4.0	0.2%
10	0	10	10.0	0.5%

[ ] = Concentration

- Four nominal concentration points range from **0.0µg/ml** to **10.0µg/ml**, which is equivalent to **0.0%** to **0.5%** of Total Free Iron Content, are subject to extraction for Total Free Iron content.

- Three standard concentration points are designed for generating a Calibration Curve.

5.11.6 Preparing Pre-Extraction Solutions for Unknown Powder Samples

5.11.6.1 Obtain the **1% Dextran Sulphate Sample Stock** Solution **(1% D.S.S)** prepared in step ***5.3.11*** for Total Free Iron test.

5.11.6.2 Pipette **8.0ml** of distilled water into a separatory funnel.

5.11.6.3 Pipette **2.0ml** of **1% D.S.S.** into the same separatory funnel as well.

5.11.6.4 The separatory funnel contains **10ml** of pre-extraction solutions of unknown powder sample and is ready to be extracted.

5.11.7 Performing Pre-Extraction for all preparations

5.11.7.1 Dispense **5ml** of **20% Hydroxylamine Hydrochloride** solution into each separatory funnel.

5.11.7.2 Dispense **10ml** of **Batho-phenanthroline** solution into each separatory funnel.

5.11.7.3 Dispense **5ml** of **10% Sodium Acetate** solution into each separatory funnel.

5.11.7.4 Place a stopper on each labelled separatory funnel, respectively and shake vigorously.

5.11.7.5 Then setting a timer allow all preparations to stand at room temperature for **exactly 20 minutes** before extraction.

5.11.8 Performing Extraction for All Preparations

5.11.8.1 When the time is up, remove the stopper and dispense **5ml** of **Chloroform** into each labelled separatory funnel, respectively.

5.11.8.2 Replace the stoppers, shake the separatory funnel vigorously to extract Free Iron from the mixtures, and then slowly open the stopper to release the pressure. Perform the shaking and releasing **three times**.

5.11.8.3 Two distinct layers form. Wait until the two layers are completely separated. Remove the stopper and drain the bottom layer (the Free Iron extraction in Chloroform) into a 100-ml volumetric flask.

5.11.8.4 Repeat steps ***5.11.8.1*** and ***5.11.8.3*** to extract each prepared sample solution **three times**.

5.11.8.5 Dilute the extraction in each 100-ml volumetric flask with **I.P.A** up to volume. Place the stoppers on and mix well by gentle hand shaking.

5.11.8.6 All extractions of unknown powder sample are for Total Free Iron content and the standard extractions with three concentration points shown below are for a Calibration Curve.

Total Vol.(ml)	Nominal [ ](µg/ml)	Equivalent [ ](%)
100	0.0	0.0% (Blank)
100	0.1	0.05%
100	0.4	0.2%
100	1.0	0.5%

[ ] = Concentration Vol. = Volume

- After the extraction, four concentration points range from **0.0µg/ml** to **1.0µg/ml**, which is equivalent to **0.0%** to **0.5%** of Total Free Iron content.

5.11.8.7 All standard point extractions are subject to the spectrophotometer measurement to generate a Total Free Iron Standard Calibration Curve.

5.11.8.8 Subsequently all unknown sample extractions are subject to the spectrophotometer measurement to determine the Total Free Iron content against the Standard Calibration Curve.

5.11.9 Measuring Absorbance by Spectrophotometer

5.11.9.1 Refer to SOP# *QC3112*

Operation and Maintenance Procedures - UV- 2101PC Spectrophotometer, Shimadzu

With Software **UV Probe** Version **1.11**

5.11.9.2 From top "**Window**" menu, Select "**2 Photometric**" mode.

5.11.9.3 From top "**Edit**" menu, Select "**Method**" to open "**Photometric Method**" sub-window.

5.11.9.3.1 Under the **Wavelength** tab, set up as:

Select - **Wavelength type: Point**
Enter - **Wavelength (λ, nm): 533.0nm**
Select - **Data Acquired by: ◘ Instrument**

5.11.9.3.2 In the **"Entries"** square, click **"Add"** to add "Wavelength type: **Point** and Wavelength (λ, nm) **533.0nm"** to finish setting.

5.11.9.3.3 Under the "**Instrument Parameters**" tab, set up as:
Select - Measuring Mode "**Absorbance**"; **Y-axis**

5.11.9.3.4 Select "**Calibration**" tab, set up as:

Select - Calibration Type "Multi Point"
Select - Formula "Fixed Wavelength"
Enter - Concentration Units "%"; X-axis
Select - Parameters Con = f (Abs)
Select - Order of Curve 1st
Un-Tick - □ "Zero Interception"

5.11.9.4 Click on "**Close**" to close the sub-window.

5.11.9.5 Fill two cuvettes with **I.P.A** and place them into the front "**Sample Beam**" compartment and one in the "**Background Beam**" compartment.

5.11.9.6 Click on **"Auto Zero"** to zero the double beams of the spectrophotometer.

5.11.9.7 On the "**Standard Table**":

5.11.9.7.1 Under the "**Sample ID**" cell, enter **Blank** and press "**Enter**".

5.11.9.7.2 Under the "**Concentration**" cell, enter the concentration **0.0%** for the **Blank** solution prepared in step **5.11.5.6**.

5.11.9.7.3 Fill the cuvette with the **Blank** solution and rinse it a few times.

5.11.9.7.4 Re-fill the **Blank** solution into the cuvette and place it into the front "**Sample Beam**" compartment.

5.11.9.7.5 Click on "**Read Std**" to obtain an absorbance reading for the **Blank** solution.

5.11.9.7.6 Under the "**Sample ID**" cell, enter a number for **the 1st standard point** and press "**Enter**".

5.11.9.7.7 Under the "**Concentration**" cell, enter the concentration in **%** for the 1st standard point, **0.05%**, prepared in step **5.11.5.6**.

5.11.9.7.8 Remove the cuvette from the front "**Sample Beam**" compartment and discard the blank.

5.11.9.7.9 Fill the cuvette with the 1st standard solution and rinse it a few times.

5.11.9.7.10 Re-fill the 1st standard solution into the cuvette and place it into the front "**Sample Beam**" compartment.

5.11.9.7.11 Click "**Read Std**" to obtain an absorbance reading for the 1st standard point.

5.11.9.7.12 Repeat step **5.11.9.7.6** to **5.11.9.7.11** entering the 2nd and 3rd standard number and **the 2nd standard point**, **0.2%** and **the 3rd standard point**, **0.5%**, respectively, to obtain absorbance readings for three standard points in measuring sequence, to generate a standard calibration curve.

5.11.9.8 On the "**Sample Table**":

5.11.9.8.1 Under the "**Sample ID**" cell, enter the batch number of the first sample solution and press "**Enter**".

5.11.9.8.2 Fill the cuvette with the first sample solution and rinse it a few times.

5.11.9.8.3 Re-fill the first sample solution into the cuvette and place it into the front "**Sample Beam**" compartment.

5.11.9.8.4 Click on "**Read Unknown**" to obtain a concentration value in **%** for the first sample solution against the standard calibration curve.

5.11.9.8.5 Remove the cuvette from the front **"Sample Beam"** compartment and discard the 1st sample solution.

5.11.9.8.6 Repeat step **5.11.9.8.1** to **5.11.9.8.5,** entering each individual batch number, to obtain all unknown sample concentrations, in **%,** in measuring sequence.

5.11.9.8.7 Remove both cuvettes and clean with distilled water and leave to dry.

5.11.9.8.8 Print out the data and graph for the Calibration Curve and the unknown powder sample.

5.11.10 Calculating Total Free Iron of Standard Points and Unknown Powder Sample

5.11.10.1 Preparation of Standard Calibration Curve Before Extracting

$$\textbf{Total Free Iron Content \%(w/w)} = \frac{C_{STD}}{C_{SMP}} \times 100\% = \frac{C_{STD}}{2000} \times 100\%$$

Where:

- C_{STD} = Nominal concentration of Total Free Iron in STD before extracting (µg/ml) **(Step 5.11.5.6)**
- C_{SMP} = Final concentration of Total Free Iron in unknown sample before extracting (µg/ml)

$$= \frac{1}{100} \times \frac{2}{10} \times 1{,}000{,}000 = \frac{1}{500} \times 1{,}000{,}000 = 2000$$

Where:
- 1 = Weight of DS40 unknown powder sample (g)
- 100 = The 1st dilution volume during sample preparation in ml
- 2 = Volume taken during sample preparation in ml
- 10 = The 2nd dilution volume during sample preparation in ml
- 1,000,000 is the conversion from g/ml to µg/ml
- 100% = Result unit conversion from µg/ml to %(w/w)

5.11.10.2 Preparation of Standard Calibration Curve After Extracting

$$\textbf{Total Free Iron Content \%(w/w)} = \frac{C_{STD}}{C_{SMP}} \times 100\% = \frac{C_{STD}}{200} \times 100\%$$

Where:

- C_{STD} = The same as **5.11.10.1** but after extracting (µg/ml) **(Step 5.11.8.6)**
- C_{SMP} = The same as **5.11.10.1** but after extracting (µg/ml)

$$= \frac{1}{100} \times \frac{2}{10} \times \frac{10}{100} \times 1{,}000{,}000 = \frac{1}{500} \times \frac{10}{100} \times 1{,}000{,}000 = 200$$

Where:
- 10 = Volumes taken for extraction preparation in ml
- 100 = Final volume for extraction preparation in ml

5.11.10.3 As Insoluble Iron concentration of the DS40 product is consistently lower than **LOQ** of Total Free Iron, there is no formula for calculation shown.

➢ When a result is lower than **0.05%**, which is the Limit of Quantitation (**LOQ**) of Total Free Iron from the method validation, it can be reported as Non-Detected (**N.D**).

5.12 RESIDUAL PYRIDINE

Sample, Reagents and Solutions
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)
Concentrated Hydrochloric Acid (HCl, 36.5 ~ 38%)
Reference Standard pH 4.0 buffer, 500ml
2% Potassium Chloride in pH 4.0 buffer (pH Electrode Storage Solution, from step **5.9.1**)
Distilled Water

Equipment and Accessories
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Calibrated pH Meter ORION STAR A111 or Equivalent
pH Electrode
Stirring Plate
Magnetic Stirring Bar
Micro-Pipette 1-ml, 0.5-ml ~ 5.0-ml (adjustable) and Tips

Glassware and Accessories
Sample Spatula
Weighing Boat / Paper
Beaker, 100-ml
Volumetric Flask with stopper, 1000-ml
Graduated Cylinder, 50-ml
Graduated Pipette, 1-ml, 10-ml
Pipette Filler
Unitary Wash Bottle
Utility Wipers
Labels

5.12.1 Preparing 0.1N Hydrochloric Acid (0.1N HCl)

5.12.1.1 Prepare a clean 1000-ml volumetric flask and fill with about 700ml of distilled water.

5.12.1.2 Pipette **8.5ml** of **concentrated HCl** solution and cautiously add it into the volumetric flask.

5.12.1.3 Dilute the solution with distilled water up to volume. Mix it well on a stirring plate with a magnetic stirring bar.

5.12.1.4 Transfer the final resulting solution into a storage bottle and label it.

5.12.2 Titrating Distilled Water as Blank

5.12.2.1 Using a 50-ml graduated cylinder, measure and transfer about **40ml** of distilled water into a 100-ml beaker. Place the beaker on the stirring plate beside the pH meter.

5.12.2.2 Press the "**Power**" button to turn the pH meter on.

5.12.2.3 Rinse the pH electrode with RO water / distilled water and wipe dry.

5.12.2.4 Immerse the pH electrode into the beaker. Mix the distilled water well with a magnetic stirring bar.

- The solution must be sufficient to cover the white ring surrounded the pH electrode, (about **2.5cm**).

5.12.2.5 Obtain a 1.0-ml graduated pipette. Suck the **0.1N HCl** prepared in step **5.12.1** up to the mark with a pipette filler.

5.12.2.6 Press "**Measure**" on the pH meter to obtain a pH value reading.

5.12.2.7 A pH reading of the distilled water is displayed while the letters "**AR**" and "**pH**" are flashing.

5.12.2.8 Start adding **0.1N HCl** into the beaker **drop by drop** to titrate the distilled water against **0.1N HCl** until a **pH of 2.8** is obtained with the "**AR**" and "**pH**" stop flashing and "**READY**" shows.

5.12.2.9 Record the volume of **0.1N HCl** solution consumed for the distilled water in the **DS** notebook.

5.12.3 Titrating Residual Pyridine in Unknown Powder Samples

- **The sample weight used for the result calculation is on a Dry Basis.**

5.12.3.1 Using a spatula, accurately weigh about **6.0000g** of **Dextran Sulphate** powder sample in a weighing boat. Then transfer it to another 100-ml beaker.

5.12.3.2 Record the powder sample weight in the **DS** notebook.

5.12.3.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.12.3.4 Continue adding about **40ml** of distilled water into the beaker as well.

5.12.3.5 Place the beaker on the stirring plate. Mix the solution well with a magnetic stirring bar until the powder is completely dissolved.

5.12.3.6 Make sure that the pH meter is still on after titrating the distilled water (Blank). Rinse the pH electrode with RO water/distilled water and wipe dry.

5.12.3.7 Immerse the pH electrode into the sample beaker and keep the sample solution mixing.

5.12.3.8 Obtain a 10.0-ml graduated pipette. Suck the **0.1N HCl** prepared in step **5.12.1** up to the mark with a pipette filler.

5.12.3.9 Press the "**Measure**" button on the pH meter to obtain a pH value reading.

5.12.3.10 A pH reading of the unknown powder sample is displayed while the letters "**AR**" and "**pH**" are flashing.

5.12.3.11 Start adding **0.1N HCl** into the beaker **drop by drop** to titrate the unknown powder sample against **0.1N HCl** until a **pH of 2.8** is obtained with "**AR**" and "**pH**" stop flashing and "**READY**" shows.

5.12.3.12 Record the volume of **0.1N HCl** solution consumed for the unknown powder sample solution in the **DS** notebook.

5.12.4 Calculating Residual Pyridine in Unknown Powder Samples

$$\text{Residual Pyridine \% (w/w)} = \frac{(\text{Volume 1} - \text{Volume 2}) \times 0.00791}{\text{Weight of DS40 Powder} - \text{LOD}} \times 100\%$$

$$= \frac{(\text{Volume 1} - \text{Volume 2}) \times 0.00791}{\text{Net Sample Weight}} \times 100\%$$

Where:

- Volume 1 = 0.1N HCl Consumed for Unknown Sample in ml
- Volume 2 = 0.1N HCl Consumed for Distilled Water in ml
- 0.00791 = 1ml of 0.1N HCl Consumed for 0.00791g of Pyridine
- Weight of DS40 Powder = Weight of Unknown powder sample (g)
- LOD = Loss on Drying in %
- Net Sample Wt.= Weight of unknown powder sample on a Dry Basis (g)
- 100% = Result unit conversion from g to % (w/w)

5.13 CLARITY

Sample, Reagents and Solutions
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)
Distilled Water

Equipment and Accessories
Calibrated Analytical Balance, A and D EJ610 or Equivalent (0.00)
Calibrated Spectrophotometer, UV-2101PC, Shimadzu or Equivalent
Spectrophotometer Cuvettes
Stirring Plate
Magnetic Stirring Bar
Fume Hood
Sterile Nitrocellulose Filter Paper, Millipore, 0.45µm
Suction Filtration Apparatus:
- Vacuum Filtration Pump
- Graduated Filtration Funnel, 300-ml
- Filter Holder - Fritted Glass Support Base & Silicone Stopper, No.8
- Graduated Erlenmeyer Filtering Flask, 500ml
- Anodized Aluminum Clamp

Glassware and Accessories
Spatula
Weighing Paper / Boat
Beaker, 250-ml
Graduated Cylinder, 50-ml, 100-ml
Pasteur Pipette
Rubber Bulb
Unitary Wash Bottle
Utility Wipers
Labels
Magnifying Glass

5.13.1 Preparing Blank and 20% Dextran Sulphate Powder Sample Solution

- **The sample weight used for the result calculation is on a Dry Basis.**

5.13.1.1 Distilled water is used as a **Blank**.

5.13.1.2 Using a spatula, accurately weigh about **20.00g** of **Dextran Sulphate** powder sample in a weighing boat. Then transfer it to a 250-ml beaker.

5.13.1.3 Record the powder sample weight in the **DS** notebook.

5.13.1.4 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.13.1.5 Continue adding about 80ml of distilled water into the beaker as well.

5.13.1.6 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder is completely dissolved.

5.13.1.7 Transfer the solution into a 100-ml graduated cylinder.

5.13.1.8 Rinse the original beaker with distilled water and add the rinsings into the graduated cylinder as well.

5.13.1.9 Dilute the resulting solution with distilled water up to volume. Mix it well by gently hand inverting and shaking.

5.13.1.10 The prepared sample solution of unknown powder is used for both the absorbance measurement by the spectrophotometer in section ***5.13.2.*** and the **Black Specks** count by filtration in section ***5.13.3.***

5.13.2 Measuring Absorbance of Unknown Powder Samples with Spectrophotometer

5.13.2.1 Refer to the SOPs# ***QC3112***

Operation and Maintenance Procedures - UV- 2101PC Spectrophotometer, Shimadzu
With Software **UV Probe** Version **1.11**

5.13.2.2 From top "**Window**" menu, Select "**2 Photometric**" mode.

5.13.2.3 From top "**Edit**" menu, Select "**Method**" to open "**Photometric Method**" sub-window.

5.13.2.3.1 Under the **Wavelength** tab, set up as:

Select - **Wavelength type: Point**
Enter - **Wavelength (λ, nm): 360.0nm**
Select - **Data Acquired by: ◘ Instrument**

5.13.2.3.2 In the **"Entries"** square, click **"Add"** to add "Wavelength type: **Point** and Wavelength (λ, nm) **360.0nm"** to finish setting.

5.13.2.4 Click on the "**Close**" to close the sub-window.

5.13.2.5 Consequently, the **Sample Table** shows **[Active]** on the screen.

5.13.2.6 Fill two cuvettes with distilled water for the **Blank** (prepared in step **5.13.1.1)** as a Reference Solution. Place one in the **"Background Beam"** compartment and one in the **"Sample Beam"** compartment.

5.13.2.7 Click on **"Auto Zero"** to zero the double beams of the spectrophotometer.

5.13.2.8 Under **Sample ID**, enter a batch number in the cell and press "**Enter**".

5.13.2.9 Take out the front "**Sample Beam**" cuvette and discard the Blank.

5.13.2.10 Rinse the cuvette with unknown sample solution, prepared in step **5.13.1.9**, and collect the rinsings into a 50-ml graduated cylinder.

5.13.2.11 Re-fill unknown powder sample solution into the cuvette and place it into the front "**Sample Beam**" compartment.

5.13.2.12 Click on "**Read Unknown**" to obtain the absorbance to determine the clarity of the unknown powder sample against the **Blank**.

5.13.2.13 Record the absorbance results in the **DS** notebook.

5.13.2.14 Take out the cuvette from the front "**Sample Beam**" compartment and transfer the measured unknown sample solution into the 50-ml graduated cylinder as well.

5.13.2.15 Repeat steps from **5.13.2.11** to **5.13.2.14** to obtain a duplicate result.

5.13.2.16 Remove both cuvettes and clean with distilled water and leave to dry.

5.13.3 Filtration of Unknown Powder Samples for Black Specks Count

5.13.3.1 Label a **0.45µm** Sterile Nitrocellulose Filter Paper with Batch No., Run No., and Date.

5.13.3.2 Prepare the filtration apparatus:
- insert the stopper surrounded filter holder into a 500-ml erlenmeyer filtration flask.
- place the labelled filter paper on the fritted glass support base of the filter holder.
- place a graduated filtration funnel to cover the filter paper.
- clamp both filter holder and graduated funnel together with an aluminum clamp.

5.13.3.3 Connect the filtration apparatus to the vacuum pump.

5.13.3.4 Turn the pump on and observe the vacuum reach about **-25Hg**. Wait until the vacuum is stable.

5.13.3.5 Combine the collected **20% DS** sample solution from the 50-ml graduated cylinder (refer to steps **5.13.2.10 & 5.13.2.14**) and leftover in 100-ml graduated cylinder together. Pour all sample solution into the filtration funnel to go through the filter paper.

5.13.3.6 When the filtration is complete, unplug the vacuum, release the aluminum clamp, and then remove the filter paper.

5.13.3.7 Place the wet filter paper on a clean background to count the number of black specks on the filter paper by using a magnifying glass.

5.13.3.8 Record the black specks results in the **DS** notebook.

5.14 CHLORIDE CONTENT

Sample, Reagents and Solutions
Dextran Sulphate Powder Sample, Mw 40,000Daltons (**DS40**)
Concentrated Nitric Acid (HNO_3, 68% ~ 70%)
0.1N Silver Nitrate ($AgNO_3$) Solution
0.1N Potassium Nitrate Solution (0.1N KNO_3)
Distilled Water

Equipment and Accessories
Calibrated Analytical Balance, A and D EJ-610 or equivalent (0.00)
Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Beckman pH/mV Meter, Φ310 Setting as mV or Equivalent
Combined Silver Ring Electrode in 0.1N KNO_3 Storage Solution or Equivalent
(with ceramic frit for argentometric titrations)
Micro-Pipette 5-ml
Micro-Pipette Tips
Stirring Plate
Magnetic Stirring Bar
Titration System: - Stand
- Automatic Self-Zeroing Burette

Glassware and Accessories
Beaker, 150-ml or 100-ml,
Volumetric flask with stopper, 500ml
Bottle and Bottle-Top Dispenser
Storage Bottle for 0.1N KNO_3 Solution
Unitary Wash Bottle
Utility Wipers
Labels
Spatula
Weighing Boat

5.14.1 Preparing 0.1N Potassium Nitrate (KNO_3) Solution for Electrode Storage

5.14.1.1 Using a spatula, accurately weigh about **5.00g** of **Potassium Nitrate** crystals in a weighing boat. Then transfer it to a 150-ml beaker.

5.14.1.2 Record the crystals weight in the Lab Reagent notebook.

5.14.1.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.14.1.4 Continue adding about 100ml of distilled water into the beaker as well.

5.14.1.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the crystals are completely dissolved.

5.14.1.6 Transfer the solution into a 500-ml volumetric flask.

5.14.1.7 Rinse the original beaker with distilled water and add the rinsings into the 500-ml volumetric flask as well.

5.14.1.8 Dilute the resulting solution with distilled water up to volume. Mix it well on the stirring plate with a magnetic stirring bar.

5.14.1.9 Transfer the final resulting **0.1N KNO_3** solution into a storage bottle and label it. Store it at room temperature in the dark.

5.14.2 Preparing Unknown Sample Solutions (Reactant)

- ***Prepare each sample in duplicate.***

5.14.2.1 Using a sample spatula, accurately weigh about **5.0000g to 5.5000g** of **Dextran Sulphate** powder sample in a weighing boat. Then transfer it to a 100-ml beaker.

5.14.2.2 Record the powder sample weight in the **DS** notebook.

5.14.2.3 Rinse the weighing boat with distilled water and add the rinsings into the beaker.

5.14.2.4 Continue adding about 50ml of distilled water into the beaker as well.

5.14.2.5 Place the beaker on a stirring plate. Mix the solution with a magnetic stirring bar until the powder sample is completely dissolved.

5.14.2.6 Dispense **2ml** of **concentrated Nitric Acid (HNO_3)** into the beaker.

5.14.2.7 Take out the Combined Silver Ring pH Electrode from **0.1N KNO_3** storage solution and rinse it with RO/distilled water.

5.14.2.8 Wipe the electrode dry and immerse it into the beaker containing DS sample solution.

5.14.2.9 Place the beaker on the stirring plate under the burette. Mix the solution with the magnetic stirring bar.

5.14.3 Titrating Unknown Powder Sample Solution

5.14.3.1 Press power button to turn on the **pH/mV** meter, setting at **mV**.

5.14.3.2 While the sample solution is mixing, observe and wait until the digital reading is stable and the "**Auto**" eye stops flashing.

5.14.3.3 Squeeze the bottle containing **0.1N Silver Nitrate ($AgNO_3$)** solution to fill the Automatic Self-Zeroing Burette just over the zero line and release it so the excess returns to the bottle.

5.14.3.4 Turn the stopcock towards the vertical position slowly to open the burette and press "**mV**" at the same time to titrate the sample solution against **0.1N $AgNO_3$** solution.

5.14.3.5 When the reading gradually reaches around **0mV**, slow down the titration by partially closing the stopcock of the burette.

5.14.3.6 Continue titrating the liquid sample **drop by drop** until a sharp potential jump shows up (usually more than 15mV).

5.14.3.7 Stop the titration at this point by completely closing the stopcock of the burette immediately.

- Response time of the electrode: after 30 seconds the indicated electrode potential should not change more than 2mV within the following 30 seconds.

- The entire titration period has to show a total potential jump of at least approximately 130 mV. The equivalence point should be in the range of 50 ~ 200 mV.

5.14.3.8 Read the volume of **0.1N $AgNO_3$** solution consumed from the burette and record it in the **DS** notebook.

5.14.3.9 Stop the mixer and remove the electrode from the sample beaker.

5.14.3.10 Rinse the electrode with RO/distilled water and place it back in the **0.1N KNO_3** storage solution.

5.14.3.11 Press power button to turn the pH/mV meter off.

5.14.3.12 Calculate the chloride content in Dextran Sulphate powder sample as in step **5.14.4**.

5.14.4 Calculating Unknown Powder Samples

$$\textbf{Chloride Content (ppm)} = \frac{\textbf{Volume of 0.1N } AgNO_3 \textbf{ x 0.003545 x 1,000,000}}{\textbf{Weight of DS40 Powder - LOD}}$$

$$= \frac{\textbf{Volume of 0.1N } AgNO_3 \textbf{ x 3545}}{\textbf{Net Sample Weight}}$$

Where:

- Volume of 0.1N $AgNO_3$ = Volume of 0.1N $AgNO_3$ consumed in ml
- 0.003545 = 1ml of 0.1N $AgNO_3$ consumed for a 0.003545g of Chloride
- 1,000,000 is the conversion factor from gram to µg (ppm)
- Weight of DS40 Powder = Weight of unknown powder sample (g)
- LOD = Loss on Drying (%)
- Net Sample Wt.= Weight of unknown powder sample on a Dry Basis (g)

6. Revision History

Revision #	Reason for Revision
03	Free Sulphate Test Updated Loss on Drying Test Updated
02	New instruments installed Operational procedures added
01	No recorded data