

Dextran Products Standard Operating Procedures

S.O.P.# QC3402-04

Supersedes: QC3402-03

Title: Testing Procedures for Dextran Powder with Different Molecular Weights

Effective Date: JUN 30 2021

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1. **Purpose**

- 1.1 This procedure is to ensure that Dextran powder with different molecular weights manufactured at Dextran Products are subject to full tests following individual test methods in the QC lab.
- 1.2 This procedure is to ensure that the quality of all tested Dextran powder with different molecular weights meet their specifications.

2. **Scope**


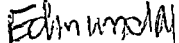

- 2.1 This procedure is applied to all Dextran powder with different molecular weights produced and marketed by Dextran Products, which are for both Final QC and Long-Term & Short-Term Stability Study testing.
- 2.2 This procedure is applied to QC Analytical Chemists for performing all testing and to 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 testing of Dextran powder with different molecular weights 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 prior to final release of the products.

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5.1 APPEARANCE

Samples

Dextran Powder Samples with different molecular weights (**DP-Mw**)

Equipment

Transparent Plastic Bag


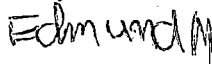

5.1.1 Performance and Observation

5.1.1.1 After spray drying, Dextran powder (DP-Mw) is sampled 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 Dextran powder sample with a specific Mw (Daltons) is a white to slightly off-white powder.

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5.2 LOSS ON DRYING (LOD)

Sample

Dextran Powder Sample with different molecular weights (**DP-Mw**)

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 Tweezers

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 **DP** notebook (**Wt. 1**).

5.2.1.3 Using a spatula, accurately weigh about **1.0000g to 1.5000g** of Dextran Powder sample with a specific molecular weight (Daltons) in each pre-weighed weighing bottle.

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

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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 **DP** notebook (**Wt. 3**).

➤ **Dried Dextran Powder is hygroscopic.**

- 5.2.2.9 Determine percentage (w/w) of Loss on Drying in Dextran 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**.

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5.2.3 *Calculating Steps of Unknown Powder Samples*

5.2.3.1 *Calculation of Loss on Drying*

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

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 the Dry Basis*

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

$$\begin{aligned} \text{Net Sample Weight on a Dry Basis} &= \text{Sample Weight (g)} - \text{LOD} \\ &= \text{Sample Weight (g)} \times (100\% - \text{LOD}\%) \\ &= \text{Sample Weight (g)} \times \text{Sample Assay}\% \end{aligned}$$

e.g. Net Sample Weight on Dry Basis (g) = Sample Weight x [100% – LOD% (4.0%)]
= 5.0 x 96.0% = 4.8

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5.3 ASH CONTENT (RESIDUE ON IGNITION)

Samples

Dextran Powder Samples with different molecular weights (DP-Mw)

Equipment and Accessories

The Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)

Crucibles

Sample Spatula

Permanent Marker

Furnace

Fume Hood

Pair of Crucible Tongs

Pair of Temperature Gloves

Desiccator with Silica Gel

5.3.1 *Preparing Unknown Powder Samples*

➤ **Prepare each sample in duplicate.**

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

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


5.3.1.2 Wearing a pair of temperature gloves and using a pair of crucible tongs, transfer the empty crucibles from the furnace into the desiccator. Cool it down to the room temperature.

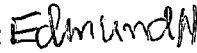
5.3.1.3 Using a pair of crucible tongs, take the empty crucibles out from the desiccator and weigh it.

5.3.1.4 Record the empty crucible's weights in the DP notebook (**Wt. 1**).

5.3.1.5 Using a sample spatula, transfer between 1.0000g to 1.5000g of Dextran powder sample with a specified molecular weight, accurately weighed, to the pre-weighed empty crucibles.

5.3.1.6 Record the total weight before heating in the DP notebook (**Wt. 2**).

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5.3.2 Heating Powder Samples

- 5.3.2.1 Using a pair of crucible tongs, place the crucibles containing the powder sample into the furnace. Turn the power on.
- 5.3.2.2 Observe the temperature going up gradually. When the temperature reaches about **810°C**, maintain such a temperature for around **10 minutes**.
- 5.3.2.3 When the time is up, turn the furnace off and wait for about **20 minutes**.
- 5.3.2.4 Wearing a pair of temperature gloves and using a pair of crucible tongs, cautiously take the crucibles out from the furnace. Immediately place them in the desiccator to allow to reach the room temperature.
- 5.3.2.5 Using a pair of crucible tongs, take the crucibles out of the desiccator. Re-weigh the crucibles containing the residue after heating. Record the weights again in the DP notebook (**Wt. 3**).
- 5.3.2.6 Determine percentage (w/w) of residue on ignition from the difference of the weights before heating and after heating. Calculate the results as equation below.

5.3.3 Calculating Residue from Unknown Powder Samples

- 5.3.3.1 A net weight of Dextran Powder sample is calculated based on the Loss on Drying (**LOD**) data, from which it then is used for the result calculation (*Refer to step 5.2.3*).


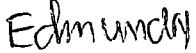

$$\text{Residue on Ignition\% (w/w)} = \frac{(\text{Wt. 3} - \text{Wt. 1})}{(\text{Wt. 2} - \text{Wt. 1}) - \text{LOD\%}} \times 100\%$$

$$= \frac{\text{Sample wt. after Heating}}{\text{Sample Wt. before Heating} - \text{LOD\%}} \times 100\%$$

$$= \frac{\text{Powder Residue}}{\text{Net Sample Wt.}} \times 100\%$$

Where:

- Wt. 1 = Weight Empty Crucible (g)
- Wt. 2 = Weight before Heating: Crucible + Sample (g)
- Wt. 3 = Weight after Heating: Crucible + Residue (g)
- LOD% = Loss on Drying in percentage
- 100% = unit conversion from g to (w/w)

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5.4 INTRINSIC VISCOSITY (IDENTIFICATION for Mw)

Sample and Reagents

Dextran Powder Samples with different molecular weights (DP-Mw)

Distilled Water

Reverse Osmosis Water (RO Water)

Equipment and Accessories

Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)

Stirring Plate

Magnetic Stirring Bar

The Water Bath Filled with Reverse Osmosis Water

The Calibrated Immersion Circulator in a Water Bath Set $37 \pm 0.1^\circ\text{C}$

Viscometer Ubbelohde # 1 (Refer to Figure1.)

Viscometer Holder

Glassware and Accessories

Weighing Dish, Polystyrene

Beaker, 100-ml

Sample Spatula

Volumetric Flask with stopper, 100-ml

Unitary Wash Bottle

Pipette Filler

Timer


5.4.1 *Preparing 1% Unknown Powder Sample Solution in Distilled Water*

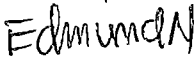
➤ The sample weight used for the result calculation is on a Dry Basis.

5.4.1.1 Using a sample spatula, accurately weigh about 1.0000g to 1.1000g of Dextran powder sample with a specific molecular weight (Daltons) in a weighing dish. Then transfer it to a 100-ml beaker.

5.4.1.2 Record the powder sample weight in the DP notebook.

5.4.1.3 Rinse the weighing dish with the distilled water and add the rinsings into the 100-ml beaker.

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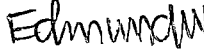

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- 5.4.1.4 Continue adding about 50ml of the distilled water into the beaker as well.
- 5.4.1.5 Place the beaker on a stirring plate and mix the resulting solution with a magnetic stirring bar until the powder is completely dissolved.
- 5.4.1.6 Transfer the resulting solution into a 100-ml volumetric flask.
- 5.4.1.7 Rinse the original beaker with the distilled water and add the rinsings into the 100-ml volumetric flask as well.
- 5.4.1.8 Dilute the resulting solution with the distilled water up to volume. Mix it well by hand shaking.

5.4.2 Measuring Efflux Time of Blank

- 5.4.2.1 Prepare a clean Ubbelohde Viscometer and a water bath filled with the Reverse Osmosis Water at 37°C.
- 5.4.2.2 Fill the viscometer with the distilled water through the tube C into the main reservoir between the two marks, H and I.
- 5.4.2.3 Cover the tube B with a finger. Using a pipette filler, suck the distilled water from the top of tube A to bring the solution level to the middle of the small bulb (D).
- 5.4.2.4 Remove the pipette filler and finger to allow the distilled water to flow freely down to rinse the walls of the viscometer.
- 5.4.2.5 Discard the distilled water after rinsing the viscometer.
- 5.4.2.6 Fill the viscometer with the distilled water through the tube C into the main reservoir between the two marks, H and I, again.
- 5.4.2.7 Place the viscometer into the holder and then into the water bath at 37°C.
- 5.4.2.8 Allow the viscometer to stand for approximately 5 minutes to equilibrate to the water bath temperature.

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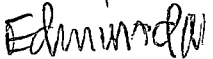

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- 5.4.2.9 Cover the tube **B** with a finger. Using a pipette filler, suck the distilled water from the top of tube **A** to bring the solution level to the middle of the small bulb (**D**).
- 5.4.2.10 Remove the pipette filler and finger to allow the distilled water 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**.
- 5.4.2.11 Record the efflux time as seconds.
- 5.4.2.12 Repeat the steps from **5.4.2.9** to **5.4.2.11**, until a stable reading is established.
- 5.4.2.13 Take the viscometer out of the water bath and discard the distilled water.

5.4.3 Measuring Efflux Time of Unknown Powder Samples

- 5.4.3.1 Fill the viscometer with **1% sample solution** through the tube **C** into the main reservoir between the two marks, **H** and **I**.
- 5.4.3.2 Cover the tube **B** with a finger. Using a pipette filler, suck **1% sample solution** from the top of tube **A** to bring the solution level to the middle of the small bulb (**D**).
- 5.4.3.3 Remove the pipette filler and finger to allow **1% sample solution** to flow freely down to rinse the walls of the viscometer.
- 5.4.3.4 Discard **1% sample solution** after rinsing the viscometer.
- 5.4.3.5 Fill the viscometer with **1% sample solution** through the tube **C** into the main reservoir between the two marks, **H** and **I**, again.
- 5.4.3.6 Place the viscometer into the holder and then into the water bath at **37°C**.
- 5.4.3.7 Allow the viscometer to stand for approximately **5 minutes** to equilibrate to the water bath temperature.
- 5.4.3.8 Cover the tube **B** with a finger. Using a pipette filler, suck **1% sample solution** from the top of the tube **A** to bring the solution level to the middle of the small bulb (**D**).

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- 5.4.3.9 Remove the pipette filler and finger to allow **1% sample 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**.
- 5.4.3.10 Record the efflux time as seconds.
- 5.4.3.11 Repeat the steps from **5.4.3.8 to 5.4.3.10**, until a stable reading is established.
- 5.4.3.12 Take the viscometer containing **1% sample solution** out of the water bath and discard the solution.
- 5.4.3.13 Wash and rinse the viscometer with the distilled water a few times.
- 5.4.3.14 After cleaning, fill the viscometer with the distilled water through the tube **C** up to mark **D** of the tube **A** and retain it in the water bath.

5.4.4 Calculation Intrinsic Viscosity of Unknown Powder Samples


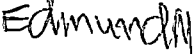

- 5.4.4.1 A net weight of Dextran Powder sample is calculated based on the Loss on Drying (**LOD**) data, from which it then is used for the result calculation (*Refer to step 5.2.3*).

Intrinsic Viscosity

$$= \frac{[(E.T. \text{ 1\% Unknown Solution } / E.T. \text{ Blank}) - 1]}{\text{Wt. of Dextran Powder - LOD\%}}$$
$$= \frac{[(E.T. \text{ 1\% Unknown Solution } / E.T. \text{ Blank}) - 1]}{\text{Net Sample Wt.}}$$

Where:

- E.T. = Efflux Time, in seconds
- Blank = Reverse Osmosis Water
- Wt. = Weight of Unknown Powder Sample (g)
- LOD% = Loss on Drying in percentage

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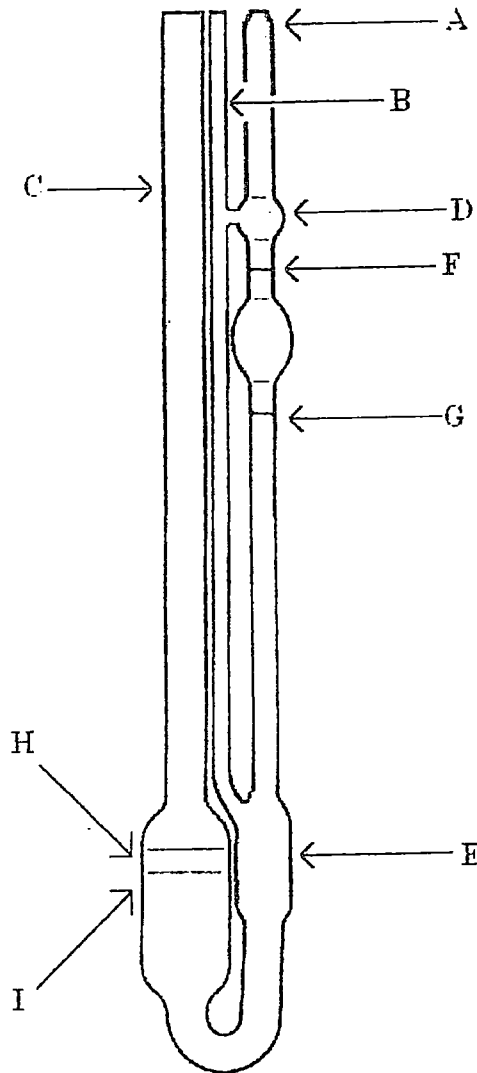


Figure 1. Ubbelohde Viscometer

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5.5 COLD WATER SOLUBILITY

Sample and Reagents

Dextran Powder Samples with different molecular weights (DP-Mw)

Distilled Water

Equipment and Accessories

The Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)

Stirring Plate

Magnetic Stirring Bar

Glassware and Accessories

Weighing Dish, Polystyrene

Beaker, 100-ml

Sample Spatula


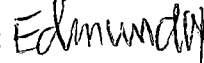

Cylinder, 100-ml

Unitary Wash Bottle

5.5.1 *Preparing Unknown Powder Sample Solution in Distilled Water*

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

- 5.5.1.1 Using a sample spatula, accurately weigh about 1.0000g of Dextran powder sample with a specific molecular weight (Daltons) in a weighing dish. Then transfer it to a 100-ml beaker.
- 5.5.1.2 Record the powder sample weight in the DP notebook.
- 5.5.1.3 Rinse the weighing dish with the distilled water and add the rinsings into the 100-ml beaker.
- 5.5.1.4 Continue adding about 50ml of the distilled water into the beaker as well.
- 5.5.1.5 Place the beaker on a stirring plate and mix the resulting solution with a magnetic stirring bar until the powder is completely dissolved.

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5.5.1.6 Transfer the resulting solution into a 100-ml graduated cylinder.

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

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


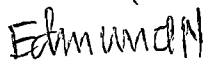

5.5.2 Observing Solubility from 1% Unknown Powder Sample Solution

5.5.2.1 Place the graduated cylinder containing **1% sample solution** on the flat counter against a white background.

5.5.2.2 Under enough brightness and against the white background, carefully seeing through **1% sample solution** and observing its solubility in the cold water.

5.5.3 Expected Result

5.5.3.1 There are no visible particles in the **1% sample solution**.

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Approved By: 	Title: QA Manager	Date: June 10, 2021

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5.6 SPECIFIC ROTATION

Sample and Reagents

Dextran Powder Samples with different molecular weights (DP-Mw)

Distilled Water

Equipment and Accessories

The Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)

Stirring Plate

Magnetic Stirring Bar

The Calibrated Automatic Polarimeter, Digital, ADP440+ or Equivalent

Polarimeter Tube, Standard Glass, Length – 1.0-dm /2.0-dm (100mm/200mm)

Glassware and Accessories

Weighing Dish, Polystyrene

Beaker, 100-ml

Sample Spatula

Volumetric Flask with stopper, 100-ml

Unitary Wash Bottle

Utility Wipers

5.6.1 *Preparing 2% Unknown Powder Sample Solution in Distilled Water*

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

5.6.1.1 Using a sample spatula, accurately weigh about 2.0000g of Dextran powder sample with a specific molecular weight (Daltons) in a weighing dish. Then transfer it to a 100-ml beaker.

5.6.1.2 Record the powder sample weight in the DP notebook.

5.6.1.3 Rinse the weighing dish with the distilled water and add the rinsings into the 100-ml beaker.

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

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- 5.6.1.5 Place the beaker on a stirring plate and mix the resulting solution with a magnetic stirring bar until the powder is completely dissolved.
- 5.6.1.6 Transfer the resulting solution into a 100-ml volumetric flask.
- 5.6.1.7 Rinse the original beaker with the distilled water and add the rinsings into the volumetric flask as well.
- 5.6.1.8 Dilute the resulting solution with the distilled water up to volume. Mix it well by hand shaking.

5.6.2 *Measuring Optical Rotation of Unknown Powder Samples*


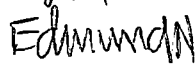

- **Allow the polarimeter 30 minutes to warm up and stabilise prior to taking readings.**
- **Keep the sample chamber empty while the instrument is initializing and not in use.**
- **To obtain a reliable data, never place a wet polarimeter tube in the sample chamber.**
- **Determine a sample optical rotation within 30 minutes of the sample preparation.**

- 5.6.2.1 Press the soft “power” key to turn the Polarimeter on – the red power indicator switches to green. Then the measurement system starts up.

Meanwhile, the stability progress bar in dark blue background is filled with the small white squares gradually from the bottom to the top, until it is fully filled.

- 5.6.2.2 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)

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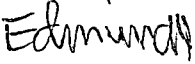

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- 5.6.2.3 After the polarimeter stabilizes, it automatically resets to zero with **0.00** displayed on the screen. If it does not, press the “**Zero**” soft key to set up the polarimeter ready for use.
- 5.6.2.4 Obtain a pre-cleaned Polarimeter standard glass tube, **1.0-dm** usually or 2.0-dm.
- 5.6.2.5 Rinse the polarimeter tube with **2% sample solution** two to three times.
- 5.6.2.6 Fill **2% sample solution** into the tube fully with avoiding to create any air bubbles.
- 5.6.2.7 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 the top of glass cover disc to close the end of the tube. It should be sealed securely, but not too tight.
- 5.6.2.8 Wipe the tube dry thoroughly, entire body and two ends with absorbent paper towel.
- 5.6.2.9 Open the lower lid of the polarimeter. Place the tube on the stainless-steel rails inside the sample chamber and close it.
- 5.6.2.10 On the screen, a flat dash line shows up immediately and the stability progress bar displays in dark blue, which identify that the zero has been carried out and it begins a new sample reading.
- 5.6.2.11 As soon as a sample optical rotation reading appears, the stability progress bar in dark blue background starts to be filled with the small white squares gradually from the bottom to the top. Meanwhile, the number reading also in dark blue background keeps going.
- 5.6.2.12 Wait until the measurement reading is stable. When the dark blue background of the stability progress bar is fully filled with the small white squares, the optical rotation reading background turns to black, indicating the measurement system obtains a result.
- 5.6.2.13 Record the measurement result in the DP notebook.
- 5.6.2.14 Open the lower lid and take out the tube from the sample chamber. Then discard the measured **2% sample solution**.
- 5.6.2.15 Repeat steps from **5.6.2.6** to **5.6.2.14** with **2% sample solution**, prepared in step **5.6.1**, to obtain duplicate readings.

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5.6.2.16 Wash and rinse the polarimeter tube with the distilled water a few times.

5.6.2.17 After cleaning, fill the tube with the distilled water to ready for next measuring use.

5.6.2.18 To switch the Polarimeter off, press the “power” soft key and hold down approximately 2 seconds until a confirmation screen shows as: “Are you sure that you want to switch off the instrument?”

5.6.2.19 Press the “Yes” button to “Standby”. The power indicator turns from green to red.

5.6.3 Calculating Specific Rotation of Unknown Powder Samples

5.6.3.1 A net weight of Dextran Powder sample is calculated based on the Loss on Drying (LOD) data, from which it then is used for the result calculation. (Refer to step 5.2.3)


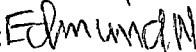

Specific Rotation $[\alpha]_D^{20}$

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

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

Where:

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

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5.7 pH Test

Sample and Reagents

Dextran Powder Samples with different molecular weights (DP-Mw)

Reference Standard pH 4.0 buffer

2% Potassium Chloride in Reference Standard pH 4.0 buffer (**pH electrode storage solution**)

Distilled Water

Reverse Osmosis Water (RO Water)

Equipment and Accessories

The Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)

Stirring Plate

Magnetic Stirring Bar

The Calibrated pH Meter ORION STAR A111 or equivalent

pH Electrode

Glassware and Accessories

Weighing Dish, Polystyrene

Beaker, 100-ml

Sample Spatula

Volumetric Flask with stopper, 100-ml


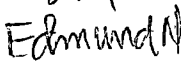

Unitary Wash Bottle

Utility Wipers

Tape label

5.7.1 Preparing pH Electrode Storage Solution

- 5.7.1.1 Using a sample spatula, accurately weigh about **2.0000g** of potassium chloride powder in a weighing dish. Then transfer it to a 600-ml beaker.
- 5.7.1.2 Record the reagent weight in the QC reagent notebook.
- 5.7.1.3 Rinse the weighing dish with **pH 4.0 buffer**. Add the rinsings into the 600-ml beaker.
- 5.7.1.4 Continue adding the rest of 500ml of **pH 4.0 buffer** into the beaker as well.
- 5.7.1.5 Place the beaker on a stirring plate and mix the resulting solution with a magnetic stirring bar until the reagent powder is completely dissolved.

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

5.7.2 Preparing Unknown Powder Sample Solution in Distilled Water

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

5.7.2.1 Preparation of Different Concentration Solutions for Different Molecular Weights

5.7.2.1.1 10% sample solution with molecular weights below & up to 40,000 Daltons

Using a sample spatula, accurately weigh about 10.0000g of Dextran powder sample with a specific molecular weight below or up to 40,000 Daltons, in a weighing dish. Then transfer it to a 100-ml beaker.

5.7.2.1.2 6% sample solution with molecular weights from 70,000 Daltons and up

Using a sample spatula, accurately weigh about 6.0000g of Dextran powder sample with a specific molecular weight from 70,000 Daltons or up, in a weighing dish. Then transfer it to a 100-ml beaker.

5.7.2.2 Record the powder sample weight in the DP notebook.

5.7.2.3 Rinse the weighing dish with the distilled water and add the rinsings into the 100-ml beaker.

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




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

5.7.2.6 Transfer the resulting solution into a 100-ml volumetric flask.

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

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

5.7.2.9 Partially transfer final resulting solution into another clean 100-ml beaker for pH measurement.

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5.7.3 Measuring pH of Unknown Powder Samples

- 5.7.3.1 Press the “**Power**” button to turn the pH meter on and wait until the letters “**AR**” and “**pH**” stop flashing and “**READY**” appears.
- 5.7.3.2 Check the solid “**READY**” and number “**2**”, “**4**”, “**7**” are displayed to confirm that the pH meter is calibrated.
- 5.7.3.3 Rinse the pH electrode with the RO water and then the distilled water.
- 5.7.3.4 Place the pH electrode into the partially transferred beaker, in which containing **10% sample solution** or **6% sample solution**, prepared in **step 5.7.2**.
 - The solution must be sufficient to cover the white ring surrounded the pH electrode, which is at least more than 1 inch /2.54cm in height.
- 5.7.3.5 Swirl or mix the **10%** or **6% sample solution** well with the electrode.
- 5.7.3.6 Press the “**Measure**” button to get pH reading.
- 5.7.3.7 The pH reading is displayed and keep changing with letters “**AR**” and “**pH**” flashing beside.
- 5.7.3.8 Wait until letters “**AR**” and “**pH**” turn to solid and “**READY**” comes on at the same time.
- 5.7.3.9 Record the pH reading in the DP notebook.
- 5.7.3.10 Repeat **step 5.7.3.6 to 5.7.3.9** to obtain a duplicate result.
- 5.7.3.11 Take out the pH electrode from the **10%** or **6% sample solution**. Rinse it completely with the RO water and then the distilled water.
- 5.7.3.12 Wipe the pH electrode dry and place it into the **Storage Solution - 2% Potassium Chloride** in Reference Standard pH 4.0 buffer.
- 5.7.3.13 Press the “**Power**” button again to turn the pH meter off.

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5.8 COLOR OF SOLUTION

Sample and Reagents

Dextran Powder Samples with different molecular weights (DP-Mw)
Distilled Water

Equipment and Accessories

The Calibrated Analytical Balance, OHAUS or Equivalent (0.0000)
Stirring Plate
Magnetic Stirring Bar
The Calibrated Spectrophotometer, UV-2101PC, Shimadzu or Equivalent
Spectrophotometer Cuvettes

Glassware and Accessories

Weighing Dish, Polystyrene
Beaker, 100-ml
Sample Spatula
Unitary Wash Bottle
Volumetric Flask, 100-ml
Utility Wipers

5.8.1 Preparing Unknown Powder Sample Solution in Distilled Water

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


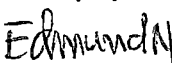

5.8.1.1 Preparation of Different Concentration Solutions for Different Molecular Weights

5.8.1.1.1 10% sample solution with molecular weights below & up to 40,000 Daltons

Using a sample spatula, accurately weigh about 10.0000g of Dextran powder sample with a specific molecular weight below or up to 40,000 Daltons, in a weighing dish. Then transfer it to a 100-ml beaker.

5.8.1.1.2 6% sample solution with molecular weights from 70,000 Daltons and up

Using a sample spatula, accurately weigh about 6.0000g of Dextran powder sample with a specific molecular weight from 70,000 Daltons or up, in a weighing dish. Then transfer it to a 100-ml beaker.

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- 5.8.1.2 Record the powder sample weight in the DP notebook.
- 5.8.1.3 Rinse the weighing dish with the distilled water and add the rinsings into the 100-ml beaker.
- 5.8.1.4 Continue adding about 50ml of the distilled water into the beaker as well.
- 5.8.1.5 Place the beaker on a stirring plate and mix the resulting solution with a magnetic stirring bar until the powder is completely dissolved.
- 5.8.1.6 Transfer the resulting solution into a 100-ml volumetric flask.
- 5.8.1.7 Rinse the original beaker with the distilled water and add the rinsings into the volumetric flask as well.
- 5.8.1.8 Dilute the resulting solution with the distilled water up to volume. Mix it well by hand shaking.
- 5.8.1.9 The final **10% sample solution** or **6% sample solution** are obtained. Then measure its absorbance with the spectrophotometer as below.

5.8.2 *Measuring Absorbance of Unknown Powder Samples with Spectrophotometer*

- 5.8.2.1 Refer to the SOPs related sections

Operating Procedure and Maintenance UV- 2101PC Spectrophotometer

- 5.8.2.2 Set up Measurement Parameters as:

5.8.2.2.1 Software **UV Probe** Version **1.11**

5.8.2.2.2 Mode: **Photometric**

- 5.8.2.2.3 • Y-axis: **Absorbance**
- X-axis: **Sample Sequence No.**

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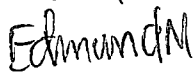

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- 5.8.2.3 From top “**Edit**” menu, select “**Method**” to open “**Photometric Method**” sub-window.
- 5.8.2.4 Inside the “**Photometric Method**” sub-window
- 5.8.2.4.1 Under the **Wavelength** tab, go for the following settings:
Select - **Wavelength type: Point**
Enter - **Wavelength (λ , nm): 375.0nm**
Select - **Data Acquired by: Instrument**
- 5.8.2.4.2 In the “**Entries**” square, click “**Add**” push button to add “Wavelength type: **Point** and Wavelength (λ , nm) **375.0nm**” to finish setting.
- 5.8.2.5 Click on the “**Close**” push button to close the sub-window.
- 5.8.2.6 Consequently, the **Sample Table** shows [**Active**] on the screen.
- 5.8.2.7 Fill two cuvettes with the distilled water as a **Blank** (Reference Solution).
- 5.8.2.8 **Zero** the double beams of the spectrophotometer following its **SOPs** procedures.
- 5.8.2.9 Under **Sample ID**, enter a batch number in the cell and press keyboard “**Enter**”.
- 5.8.2.10 Take out the “**Sample Beam**” cuvette and discard the distilled water.
- 5.8.2.11 Pour **10%** or **6% sample solution** into the cuvette and rinse it a few times.
- 5.8.2.12 Re-fill **10%** or **6% sample solution** into the cuvettes and place it into the front “**Sample Beam**” compartment.
- 5.8.2.13 Click on the “**Read Unknown**” push button to determine the Optical Density for the unknown sample the against the Blank.
- 5.8.2.14 Record the absorbance results in the DP notebook.
- 5.8.2.15 Take out the cuvette from the front “**Sample Beam**” compartment and discard the measured **10% or 6% sample solution**.

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5.8.2.16 Repeat steps from **5.8.2.12** to **5.8.2.15** to obtain a duplicate result.

5.8.2.17 Clean the cuvettes with distilled water and leave to dry.

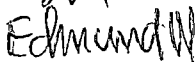
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5.9 MOLECULAR WEIGHTS AND DISTRIBUTION BY GPC ANALYSIS

5.9.1 Introduction

The Molecular weight of Dextran Powder raw material is determined by comparison with a standard curve, prepared from seven (7) Dextran Standards with exactly known molecular weight, using gel permeation chromatography (GPC) combined with a Refractive Index (RI) as detector.

5.9.2 Description of Method

5.9.2.1 Equipment


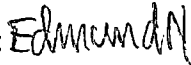

- Analytical Balance
- Magnetic Stirrer
- pH Meter
- HPLC system:
Agilent 1100 Series equipped with a RI detector and GPC Data Analysis Software for HP Chemstation data processing, or equivalent
- Column: TSK Gel, G5000 PWXL (10 μ m), 30cm x 7.8mm ID (Tosohaas)

5.9.2.2 Supplies

- HPLC solvent filtering apparatus and 0.22 micron filters
- Magnetic stir bars
- Beakers and filling funnels
- Volumetric flasks

5.9.2.3 Reagents

- HPLC grade water
- Sodium Sulfate Anhydrous, Fisher, or equivalent
- Sodium Phosphate Monobasic Anhydrous, A&C, or equivalent
- 1 N NaOH (to adjust pH)
- Sodium Azide, Sigma or equivalent

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5.9.2.4 Standards

5.9.2.4.1 The Standards are stored at room temperature.

5.9.2.4.2 The following Dextran Reference Standards (Fluka Chemie) are used to prepare the Standard Curve. The corresponding **Mp** value may be found on the certificate of analysis of each Dextran Reference Standard.

<u>Mw (approx. D)</u>	<u>Mp (Certificate of Analysis)</u>
25,000	21,400
50,000	43,500
80,000	66,700
150,000	123,600
270,000	196,300
410,000	276,500
670,000	401,300

5.9.3 Preparation of Mobile Phase

- *The whole analysis, including standard curve and samples, must be analyzed using the same batch of mobile phase. Furthermore, standard and samples must be prepared with that same batch of mobile phase.*

5.9.3.1 Dissolve 17.8 g of Sodium Sulfate Anhydrous and 0.276 g of Sodium Phosphate Monobasic in 1000ml of water (or any weights and volume respecting this ratio).




5.9.3.2 Adjust the pH of the solution to 6.0 with a solution of NaOH 1N.

5.9.3.3 Filter through a 0.22µm filter and degas before use.

5.9.4 Preparation of Dextran Standards

- *Dextran Standard Preparations are stable for 72 hours at room temperature.*

5.9.4.1 Into separate HPLC vials, accurately weigh approximately 5mg (\pm 0.3mg) of each Dextran Standard.

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5.9.4.2 Add 1.0ml of mobile phase and mix gently by inversion (do not vortex).

Once diluted, allow the standards to stand at room temperature for at least 2 hours before injection.

5.9.5 Sample Preparation (Perform each sample preparation in duplicate)

➤ **Sample preparations are stable for 36 hours at room temperature.**

5.9.5.1 Into a 25ml volumetric flask, accurately weigh approximately 125mg of Dextran Powder raw material (or any weights and volume respecting this ratio to obtain the same concentration).

5.9.5.2 Complete to volume with mobile phase and mix well to obtain a known final concentration of 5mg/ml of Dextran Powder raw material

➤ **Let the sample preparation stand at room temperature for at least 2 hours before analysis.**

5.9.6 Chromatographic Conditions

5.9.6.1 Column Conditioning

5.9.6.1.1 The column is stored at ~4°C (in fridge) in a 0.05% Sodium Azide Solution.

5.9.6.1.2 Before each analysis, condition the column first with water at a flow rate of 0.4 ml/min for at least 30 minutes and then with mobile phase at a flow rate 0.4 ml/min for at least 2 hours.


5.9.6.2 Column Storage and System Wash

5.9.6.2.1 When analysis is completed, rinse the column with water at a flow rate of 0.4ml/min for at least 30 minutes.

5.9.6.2.2 Rinse the column with 0.05% Sodium Azide Solution at a flow rate of 0.4 ml/min for at least 30 minutes.

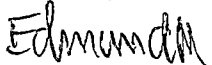
5.9.6.2.3 Take off the column. The GPC column is stored at ~4°C in a 0.05% Sodium Azide Solution.

5.9.6.2.4 Install a bypass and rinse the system (pump and RID) with filtered HPLC grade water.

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5.9.6.3 General Notes

- *The column and detector temperature must remain stable during analysis. This is to avoid retention time and fluctuation.*

Detector: Refractive Index (RI)
Polarity: Positive
Automatic Recycling after analysis: on
Detector temperature: 25° (heater on)

Column: TSK Gel, G5000 PWXL (10µm)
30cm x 7.8mm ID (Tosohaas)

Column Temperature: 25°C

Flow Rate: 0.6 ml/min (about 250psi or 18bar)
(Never exceed 1.0mL/min)

Injection volume: 10 µl

Mobile phase: Sodium Sulfate Anhydrous 17.8g
Sodium Phosphate Monobasic 0.276g
HPLC Grade Water 1000ml
(All adjusted to pH with 1N NaOH)

Total Run Time: 32min. for standards and sample preparations

5.9.6.4 Typical Retention Times (RT) VS Dextran Standard Solutions

<u>Mw (Approx. D)</u>	<u>RT (Minutes)</u>
25,000	14.9 min
50,000	14.1 min
80,000	13.5 min
150,000	12.9 min
270,000	12.4 min
410,000	12.0 min
670,000	11.5 min

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5.9.7 System Suitability

5.9.7.1 Procedures

- 5.9.7.1.1 Equilibrium of the column with mobile phase at 0.4ml/min for at least 2 hours under the conditions in Section **5.9.6.1** in order to obtain stable retention times.
- 5.9.7.1.2 Perform one (1) injection of mobile phase blank and Dextran Standard Solution 150000 D (Mw) in order to condition the column.
- 5.9.7.1.3 To determine the reproducibility (RSD), perform a total of five (5) replicate injections of the Dextran Standard Solution Mw 270,000D prepared in Section **5.9.4**.

5.9.7.2 System Suitability Criteria

- 5.9.7.2.1 The system suitability parameters are calculated as follows:

The reproducibility of the Dextran Standard Solution Mw 270,000D **Retention Time** from five replicate injection should be less than or equal to 0.5% (**RSD \leq 0.5%**).


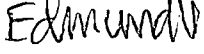

5.9.8 Analysis

- 5.9.8.1 Once the system suitability is completed by following Section **5.9.7**, perform injections as follows:

- 5.9.8.1.1 Mobile phase blank (may be injected before system suitability).
- 5.9.8.1.2 Dextran Standard Solutions starting from Mw 25,000D to Mw 670,000D (Section **5.9.4**)
- 5.9.8.1.3 Sample Preparations (not more than 6 sample preparations)^[1]

^[1] Inject the Dextran Standard Solution Mw 270,000D every six (6) sample preparations and complete the sequence with a last injection of Dextran Standard Solution Mw 270,000D.

- 5.9.8.1.4 Dextran Standard Solution Mw 270,000D.

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5.9.8.2 The retention time of each Dextran Standard Solution Mw 270,000D injected during the sequence must not differ by more than 0.10min from the average retention time of the five (5) replicate injections of the Dextran Standard Solution Mw 270,000D from the system suitability.

5.9.8.3 Determine the Correlation coefficient (R^2) of the Standard Curve as described in Section **5.9.9**.

5.9.8.4 Calculate the Molecular Weight (Mw, Mn, Polydispersity, and Mass Distribution with 10% High-fraction and 10% Low-fraction in the sample of Dextran Powder raw material as described in Section **5.9.9**.

5.9.9 Calculation

5.9.9.1 Using the GPC Data Analysis Software for HP Chemstation data processing, plot the retention time of the Dextran Standards versus the corresponding **Mp Values (these values are found in the certificate of analysis of each Dextran Standard)**.


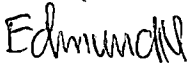

5.9.9.2 The equation used is Polynomial order 2. The GPC Data Analysis software automatically calculates the *elution volume (mL)* as well as the *Molecular Weight* of the studied sample.

5.9.9.3 Standard Curve: Plot the **elution volume (ml)** versus the **\log_{10} Mp** for each Dextran Standard using excel worksheet. The polynomial equation (Order 2) which best-fitted data is used to generated the standard curve equation. The Correlation coefficient R^2 should be equal or greater than 0.9970.

5.9.9.4 The Molecular Weight (Mw, Mn and Polydispersity) and Mass Distribution of Dextran Powder raw material are calculated by the GPC Data Analysis software.

5.9.10 Reporting of Results

5.9.10.1 Report the Molecular Weight (Mw, Mn, Polydispersity) and Mass Distribution with 10%High-fraction and 10%Low-fraction of Dextran powder raw material.

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6. Revision History

Revision #	Reason for Revision
04	<ul style="list-style-type: none">• LOD procedure was updated
03	<ul style="list-style-type: none">• Updated format• Changed DI water to RO water
02	<ul style="list-style-type: none">• The content of the SOPs is established.• The format of the SOPs is established.• Revised from 3402-01 “ Testing Procedure for Dextran Powder for Procyte”
01	<ul style="list-style-type: none">• 3402-01 “ Testing Procedure for Dextran Powder for Procyte”

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