

CPS Disc Centrifuge Quick User Guide - E

Start-up

Installation of the detector:

For safe transport the CPS Disc Centrifuge comes with an uninstalled light source detector assembly. Therefore the first step will be installation of the detector.

Procedure:

- Unscrew the injector port in the door (clockwise direction).
- Push door to the right and remove from the system.
- Insert the detector. A guide is set to ensure the correct distance to the door and to the disc is maintained.
- Connect the wiring between the detector and the instrument.
- Push the detector to the left until it comes up against the stop.
- Bolt the detector firmly into position.
- Slowly turn the disc by hand and ensure that it does not touch the detector (particularly we must avoid any scratching of the disc surface!)
- Replace the door and push to the left to close it.
- Replace the injector port.

Starting the CPS software and preparation for the first measurement:

Please make sure that in the PC operating system the point and comma are adjusted to US English!

- Start the CPS Disc Centrifuge software.
- In the menu chose option DEFINE PROCEDURE and input the required parameters and values (probably your samples were already measured at a demonstration in our laboratory and the necessary parameters and values are readily available. Feel free to consult with us at any time for new suggestions regards parameters for any new sample procedures). There is also a reference to the recommended disc centrifuge speed (rpm) in the options menu, this is calculated automatically according to the values that have been input. However, the indicated value is somewhat conservative and can usually easily be exceeded by 10%-20%. Once the procedure is set up it should be stored under a new name.
- Now go to CHOOSE PROCEDURE and change to the newly created procedure.
- In the main menu we suggest you set the speed control to MANUAL. You can then set the required speed in rpm using the slider control. As mentioned earlier, this can usually be set 10%-20% higher than the automatically calculated value.
- Click START to start the disc centrifuge.
- As soon as the set speed is reached you are ready to begin production of the gradient.

The density gradient:

For aqueous dispersions a gradient made from Sucrose or by Dextran is usually used. Typical gradients are rising from 2% sugar solution to 8% sugar solution, from 4% to 12% or from 8% to 24%. Probably your samples were already measured at a demonstration or in our laboratory, or we have already discussed your application. Therefore you should already have a good indication of which gradient to use. Supplied with the instrument is an assortment at gradient liquids of different density. Of course the production of a sugar gradient liquid is also possible at any time in your own laboratory. If you have new sample materials you can consult with us at any time in order to obtain suggestions for which gradient should be used. There are two possibilities regards constructing your gradients.

Gradient Build Option 1:

Use separate solutions in order of decreasing sugar concentration (e.g. as per bottles supplied with the instrument).

Example:

24%/22%/20%/18%/16%/14%/12%/10%/8%

Now, using a syringe, you take up from the first bottle (24% solution), 2 ml (1.8 or 1.6 ml, according to our suggestion) and inject this into the disc (already rotating at the set analysis speed). Proceed in the same manner with the subsequent bottles (22% to 8%) in the order as follows.

Example:

24% 1.8 ml
22% 1.8 ml
20% 1.8 ml
18% 1.8 ml
16% 1.8 ml
14% 1.8 ml
12% 1.8 ml
10% 1.8 ml
8% 1.8 ml

Finally you inject approximately 0.5 ml of Dodecane to extend the lifetime of the gradient by limiting evaporation.

A waiting period of approximately 10 to 15 minutes should now be sufficient for the created "step" gradient to become linear.

Gradient Build Option 2:

Use only bottles with the highest and the lowest density required, thus the highest and lowest sugar concentration.

Example:

24% and 8%

Now, using a syringe, you take up from the first bottle (24% solution), 2 ml (1.8 or 1.6 ml, according to our suggestion) and inject this into the disc (already rotating at the set analysis speed). Next, the syringe is filled with only 1.8 ml (1.6 or 1.4 ml) of the 24% solution and filled up with 0.2 ml of the 8% solution, shaken and injected into the disc. Next 1.6 ml (1.4 or 1.2 ml) and 0.4 ml, then 1.4 ml (1.2 or 1.0 ml) and 0.6 ml, and so on until the last syringe consists of only 2 ml (1.8 or 1.6 ml) of 8% solution.

Example:

24% 1.8 ml	8% 0 ml
24% 1.6 ml	8% 0.2 ml
24% 1.4 ml	8% 0.4 ml
24% 1.2 ml	8% 0.6 ml
24% 1.0 ml	8% 0.8 ml
24% 0.8 ml	8% 1.0 ml
24% 0.6 ml	8% 1.2 ml
24% 0.4 ml	8% 1.4 ml
24% 0.2 ml	8% 1.6 ml
24% 0 ml	8% 1.8 ml

Finally you inject approximately 0.5 ml of Dodecane to extend the lifetime of the gradient by limiting evaporation.

A waiting period of approximately 10 to 15 minutes should now be sufficient for the created "step" gradient to become linear.

Dodecane:

With aqueous gradients which are set up with Sucrose or Dextran, it is important that you inject approximately 0.5 ml of Dodecane after the final lowest density gradient solution has been injected, in order to extend the life time of the gradient by limiting evaporation. Please consider that you should not use a syringe with rubber for Dodecane.

The first measurement:

Since the procedure and the density gradient are complete, the first measurement can now take place.

- Click OPERATE ANALYSER. Please follow the references in the command line in this program window.
- Enter sample name.
- When prompted inject 100 µl of the appropriate calibration standard.
- When prompted inject 100 µl of the appropriate sample.
- Observe the absorption curve provided during the measurement. The graphs in green, blue and red show the detector signal with different levels of attenuation. The red graph line should never go beyond the 80% value, if it does then the sample concentration is too high and further dilution is required.
- During the measurement you can always click on EXIT and go back into the program without terminating the measurement, for example to view some previously collected data. With TERMINATE you will terminate the measurement at that point and the data is saved and can be evaluated in the usual way. With ABORT data will be lost! Please consider that during terminated or aborted measurements, all particles must already have gone past the detector before a new calibration or measurement can start. Otherwise these particles may interfere with subsequent measurements.

Cleaning:

After completion of all measurements for the day, or with a specific gradient, the system is stopped and the disc ceases rotation. Once the disc has stopped rotation the door can be opened by sliding it to the right. The disc can remain installed in the instrument for cleaning.

- Remove the cover cap of the disc using the special tool provided.
- Suck out the liquid with a water-operated vacuum pump, a plastic syringe, or a flexible pipette from the disc. Please never use any device with a metal or a glass tip since these can scratch the disc.
- Use a lint free cloth or paper towel, insert it into the disc, rotate and clean.
- Place water into the disc to flush, remove as before, rotate and clean again.
- Use a further cloth or paper towel to clean the disc intensively, pressing the cloth or paper towel right inside and against the disc surfaces as the disc is manually rotated. Depending upon cleanliness of the cloth or paper towel after this procedure, it should be again repeated. Note that if a coloured sample has been used then you will see this on the cloth or paper towel as you clean, and the procedure should be repeated until no further contamination is visible on the cleaning cloth or paper towel.
- Finally a clean cloth or paper towel should be soaked with ethanol or isopropanol and clean again as already described. The exterior of the disc (finger marks) in front and at the back should also be cleaned.

When not in use:

If you do not use the system over night, you should leave it with the disc cleaned, not rotating, instrument switched on and with the software program shut down. Switching the overall system off is not recommended.

If the system is likely to be left for a longer time period, e.g. over the weekend or longer, you should also leave it as described above, but you may wish to switch off the instrument system. However, in this case please consider that when restarted after such an extended period, a "warming up phase" of approximately 1 hour will be necessary for optimum performance. It is not necessary for the disc to be in rotation during such a warm up phase.

Changing a Disc:

Procedure:

- Unscrew the injection port (in a clockwise direction) and remove from the door.
- Push door to the right and remove from the system.
- Loosen the detector with the Allen key, pull away to the right past the mark.
- Use the fork wrench on the axle behind the disc to loosen it. Then hold the disc firmly with one hand and push the fork wrench downward in a clockwise direction (or upwards if more convenient). Thus the disc is loosened and can be unscrewed by hand in a clockwise direction.
- Place the new disc in position and reverse the above procedure finally tightening with the fork wrench. There is no need to over tighten since by it's rotation the disc becomes tightened optimally during operation.
- Now replace the detector. A guide behind helps set the detector in the correct position. Ensure the correct distance to the door and to the disc is maintained.
- If the wiring to the detector was removed then replace this.
- Push detector to the left towards the disc until it reaches the stop screw.
- Bolt the detector firmly in position with the Allen key.
- Slowly turn the disc by hand to ensure no contact with the detector!
- Replace the door push to the left.
- Replace the injection port.

Filing:

Please do not use Windows file manager for working on, deleting or copying CPS files! The CPS program often counts the files with the measurement sequence and data could easily become out of synchronization or corrupted by doing this. Please use only the CPS file manager for all operations regards deletion, export, import or copying of CPS files.

Gradients:

- Gradients should be chemically compatible with the sample, i.e. samples which are dispersed in solvents require a gradient with compatible solvents. A typical solvent gradient might be toluene and MEK for example. Since these gradients are very infinitely variable, you may wish to consult us for advise on which gradient may be most suitable for a new application.
- It is fundamental that the highest density layer in any gradient should be lower in density than the particles in the sample, otherwise particles may never arrive at the detector!
- Wherever possible use a buffer or solvent that is the same as that in which the sample is dispersed.
- Particles of higher density tend to require gradients with larger density variations (a "steeper" gradient). Polystyrene for example can be measured with that 2%/8% gradient, whereas diamond particles would require the 8%/24% gradient.
- Dextran is useful for larger and/or even more dense particles since it has a higher molecular weight and a higher viscosity. A Dextran gradient remains stable for longer than a Sucrose gradient. The noise also becomes lower when using Dextran, the analysis time however increases. Naturally it must be considered that with low density particles they must be able to actually penetrate the Dextran gradient.