JoVE: Science Education Working with Centrifuges --Manuscript Draft--

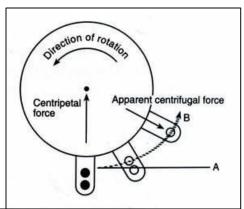
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Overview

Earth's gravitation force is capable of separating heterogeneous mixtures. However, many systems avert this type of separation owing to the length of time usually required in such cases. Centrifugation is one of the most powerful tools for the separation of heterogeneous mixtures.¹ It involves the application of centripetal force for sedimentation of the heavier phase leading to separation of the two phases and is a commonly used technique in industrial and laboratory setting.



In a typical centrifugation process, particles suspended in a fluid are spun about an axis of rotation so that the influence of the force acting away from the axis drives the particles towards the bottom of the vessel at a rate, dependent on the size and density of the particles. Centrifugation is therefore a technique to speed up the process of sedimentation.

To ensure safety in the usage of a centrifuge, the user should be properly trained and made aware of the

hazards that might result from its usage. The following sections present a discussion of the handling and safety while working with centrifuge.

Principles

Fig. 1.

A centrifuge consists of three basic parts—a rotor, a drive shaft, and a motor.² The rotor

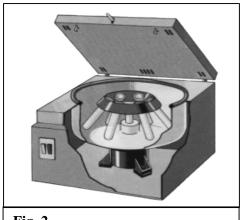


Fig. 2.

holds the tubes, bottles, or bags containing the liquids to be centrifuged. It is usually made of a high strength material such as an aluminum alloy or stainless steel. Different rotor types and sizes, interchangeable with one another, can be mounted on the drive shaft, which connects to the motor. The motor provides the power to turn the rotor. Usually, a secure cabinet surrounds and supports these parts. The operating controls and indicator dials for speed and time are mounted on the cabinet. Most centrifuges have a brake system to bring the rotor to a standstill shortly after the run is finished.

Many centrifuges are also refrigerated to prevent delicate biological samples from getting warm since samples tend to heat up during the centrifugation process. There are both low-speed and high-speed instruments for centrifugation. Low-speed centrifuges have maximum rotor speeds of less than 10,000 rpm while high-speed instruments are capable of speeding up to 21,000 rpm. Ultracentrifuges can exceed rotation speeds of 30,000 rpm.

Due to the high speed at which a centrifuge operates, it can cause serious injury to the user if mishandled. The hazards caused by centrifugation is connected primarily either to mechanical failure or dispersion of aerosols. Due to the centrifugal force the rotor experiences a load, causing stretching or a change in the dimensions of the metal. Each rotor is designed to withstand a certain amount of stress and return to its original dimensions. However, if that amount of stress is exceeded, the rotor will not return to its original shape and size. This causes minute cracks and other wear that will deteriorate the rotor over time, leading to possibly dangerous consequences. Occasionally samples may spill or leak inside the centrifuge due to failure of the rotor or associated centrifuge parts. Anytime a sample containing hazardous materials leak inside the centrifuge, it can pose potential hazards to the user and others present in the surrounding area. Hence, it is important for all users to follow standard steps for the safe operation of centrifuges and be trained to handle situations in case of operation failure or other hazards. The user should never become complacent when working with low-speed instruments. They can still cause significant injury to the user.

Safe procedure for operation of centrifuge

1. General Protocol for Centrifuge Usage

- 1.1. The lab supervisor should ensure that all centrifuge users are properly trained in the selection, care, and use of centrifuge.
- 1.2. Any new user should be closely supervised by trained user until he/she is confident on how to properly use the instrument.
- 1.3. Prior to using the instrument, each user should carefully read and understand the safety instructions for the specific centrifuge.
- 1.4. Depending on sample, compatible rotors should be used.
- 1.5. The rotor, tube, and spindle should be clean, dry, and free from any crack or deformities.
- 1.6. The O-rings should be checked for any wear or cracks.

- 1.7. It is important to make sure that the tubes used for centrifugation is compatible with the sample and the rotation speed (low, high, or ultra).
- 1.8. The centrifuge tubes should not be filled more than three-fourths of the maximum fill level.
- 1.9. The tubes should be capped tightly.
- 1.10. It is extremely important to balance the tubes opposite to each other. Failure to do so may lead to incomplete separation and in the extreme case can cause dispersion of the tube contents in the housing of the centrifuge.
- 1.11. Before turning on the centrifuge the rotor should be checked to ensure it is correctly seated on the drive and the lid is properly closed.
- 1.12. The lid should be kept closed at all times when the centrifuge is in operation and should never be open until the rotor completely stops.
- 1.13. The user should not leave the centrifuge until the desired operation speed is attained and the machine is running safely at full maximum speed. In case of unusual sound or vibration, the instrument should be immediately turned off. The speed limit of the instrument should never be exceeded. Upon turning off, allow sufficient time for the rotor to slow down to a stopped position. Modern centrifuges have indicators that will signify that it is safe to open the centrifuge.
- 1.14. Once the centrifugation is over, samples should be removed and the instrument should be checked for any spills or leaks. If necessary, the rotor should be cleaned thoroughly.

2. Additional Precautions While Handling Hazardous Chemicals or Infectious Materials

- 2.1. When using hazardous chemicals or biological samples that might be infectious, it is important to label the instrument while in usage to alert other workers to the danger.
- 2.2. Appropriate gloves¹ should be worn to handle hazardous samples.
- 2.3. Sealed safety tubes should be used. It is preferable to use secondary confinement if available for the centrifuge tubes. These are usually in the form of plastic or glass inserts that are placed directly in the sample buckets of the centrifuge.
- 2.4. Once the centrifugation is over the tubes should be removed from the rotor and opened only in fume hood or biological safety cabinet as applicable.

- 2.5. Cleaning, if required, should be done with extreme care so as to not expose the operator to the hazardous chemicals or infectious materials. In case of spills of such samples see *Emergency Situations* below.
- 2.6. For specific type of hazardous samples it is preferable to maintain standard procedure and cleaning protocols in laboratory. These should be reviewed and updated accordingly as necessary.

3. Emergency Situations and Their Safety Measures

An emergency situation may arise if the rotor malfunctions or if there is a spill in the centrifuge due to tube breakage or mishandling. In such situations the following steps should be taken.

- 1.1. The centrifuge should be turned off immediately and the lid should be kept closed until others are notified and evacuate the location. The power should be terminated at the power button on the instrument. The button will be located either on the front, side, or back of the instrument. The user should familiarize themselves with its location. If the power button is malfunctioning, then the user should remove the main power plug from the wall socket.
- 1.2. To reduce the risk of aerosols, it is sometimes better to leave the centrifuge untouched for at a 0.5 h. Consult with the centrifuge technical document.
- 1.3. For cleaning up spills, the operator should be well acquainted with the sample being used and should wear proper attire including lab-coat, gloves, facial shield, and safety glasses.
 - 1.3.1.Once cleaned, the spilled material should be properly disposed and the contaminated protective clothing should be disposed of or cleaned properly. Hands and any exposed skin should be washed thoroughly.
- 1.4. If overtly exposed to highly hazardous materials, 911 should be called or immediate medical attention should be administered to the afflicted person.

4. Maintenance of Centrifuge

The body of the centrifuge is usually made from metals that can corrode in the presence of moisture, chemicals, or strong cleaning agents. For proper maintenance of the centrifuge, it is recommended to carefully follow manufacturer instructions for cleaning and maintenance. Other than that, a few general measures for maintenance to be kept in mind are as follows.

- 4.1. The centrifuge should always be kept clean and dry.
- 4.2. Any kind of spills should be immediately cleaned.

- 4.3. The rotor should be decontaminated after use with biological and radioactive materials. It is recommended to use 10% bleach for 30 min followed by 70% ethanol and let it air dry.
- 4.4. Abrasive brush wires should never be used to clean rotor and associated parts.
- 4.5. The rotor should be inspected after each use by the operator and at least annually by manufacturer inspector.
- 4.6. A log book should be maintained for each instrument to keep a record of the service history, warranty, and warranty expiration dates.

Conclusion

With increased usage of centrifuges in laboratory and industry, the risks of hazards for using centrifuges also increases. However, careful handling of the apparatus and proper knowledge of its use and safety measures can mitigate accidents to a great deal. A basic guideline of working with centrifuges is provided here. Nonetheless, it may vary marginally depending on the particular centrifuge machine used. Hence, it is always recommended to carefully read and understand the user manual of the particular machine before starting to use it.

Legend

Figure 1. Different forces acting during centrifugation¹

Figure 2. A typical centrifuge setup²

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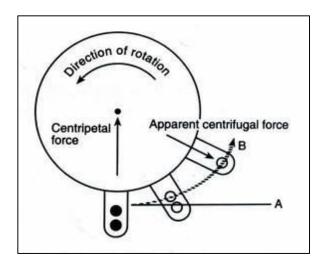
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Figure 1



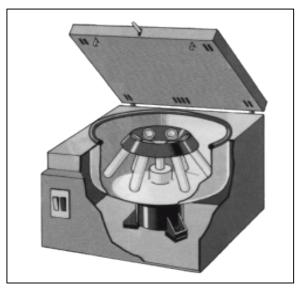


Figure 2