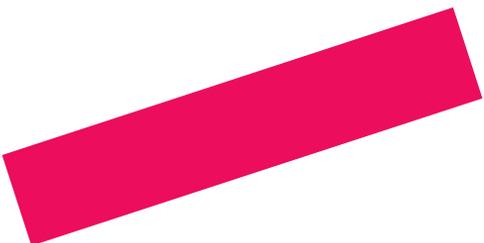ATBC – Biomoleculen (BMBIO-T)

Molecular and Biochemical Techniques

Lesson 2: Centrifugation

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Program Molecular and Biochemical Techniques

Lesson 1: Electrophoresis

Lesson 2: Centrifugation

Lesson 3: Fluorescence

Learning goals lesson 2: Centrifugation

After this lesson students:

- Can name different types of centrifugation

- Are able to explain the principle of centrifugation by explaining sedimentation, gravitational force, sedimentation velocity and acceleration.

- Know the difference between RCF and RPM, understand how the formula's are build up and are able to calculate from one to another.

- Are able to argument how an experiment can be optimized, based on given formula's.

The purpose of centrifugation

The purpose is to separate non-dissolved substances or particles based on differences in size, density, viscosity of the medium, and the rotor speed.
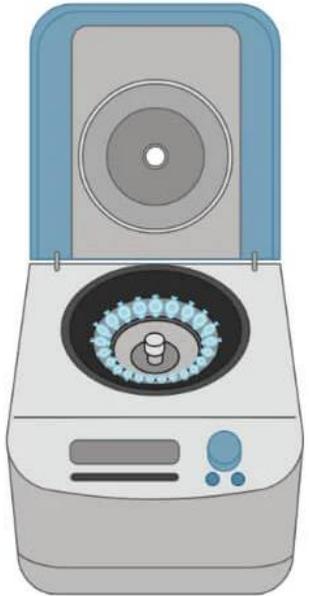
- **How does a Centrifuge work?**

A centrifuge works by rotating at rapid speeds, thereby separating substances.

- **What is sedimentation?**

Under the influence of gravitational (g-force) and centripetal force substances separate according to their density.
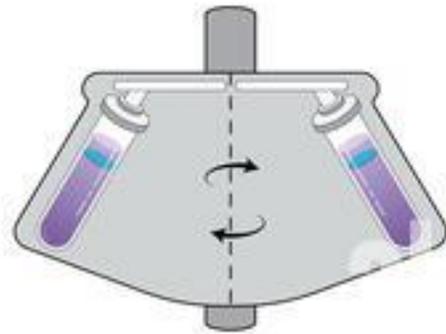
Types of centrifuges
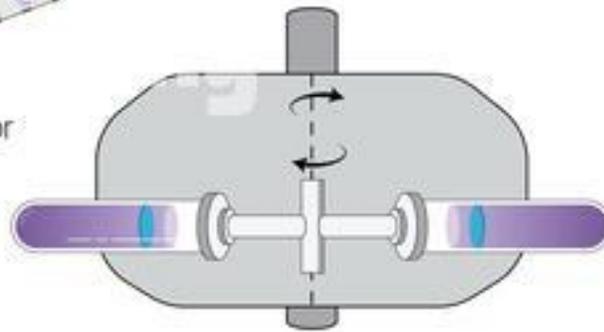

Microcentrifuge

Benchtop or Tabletop centrifuges

Ultracentrifuges

Microbe Notes. (n.d.). *10 types of centrifuge with principles and uses*.
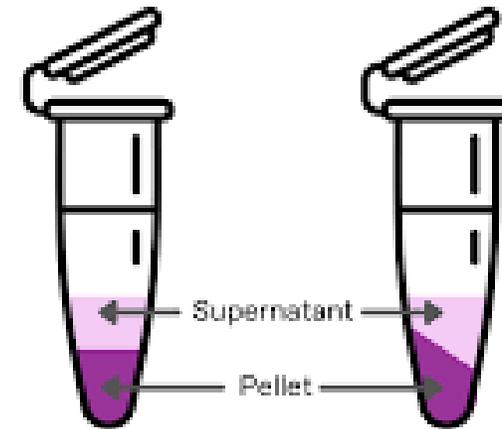Retrieved January 17, 2025,
from https://microbenotes.com/types-of-centrifuge/

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Types of centrifuges: Fixed angle vs swinging bucket rotor

Fixed Angle Rotor

Swing-out bucket rotor

Richmond scientific. (March, 2022). *Fixed Angle vs. Swing Out Rotors: What's the difference?*
Retrieved January 27, 2025, from https://www.richmondscientific.com/difference-between-
fixed-angle-and-swing-out-centrifuge-rotors

Supernatant

Pellet

Swing out rotor   Fixed angle rotor

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Types of centrifuges: speed depicted in RPM or RCF

RCF: The Relative Centrifugal Force
RPM: Revolutions Per Minute (RPM)

In this course and in your later work it is important to know the difference and how to switch from one to another!

Why?
Spinning blood samples is usually done at 2300 RCF.
What will happen when you use a (much) higher g-force?

What is the difference between RCF and RPM?

RPM measures how fast the rotor is
RCF measures the G-force (gravitational force) the sample is subject to.

→ RCF accommodates the rotor size into its measurement so we can determine the gravitational force being applied.
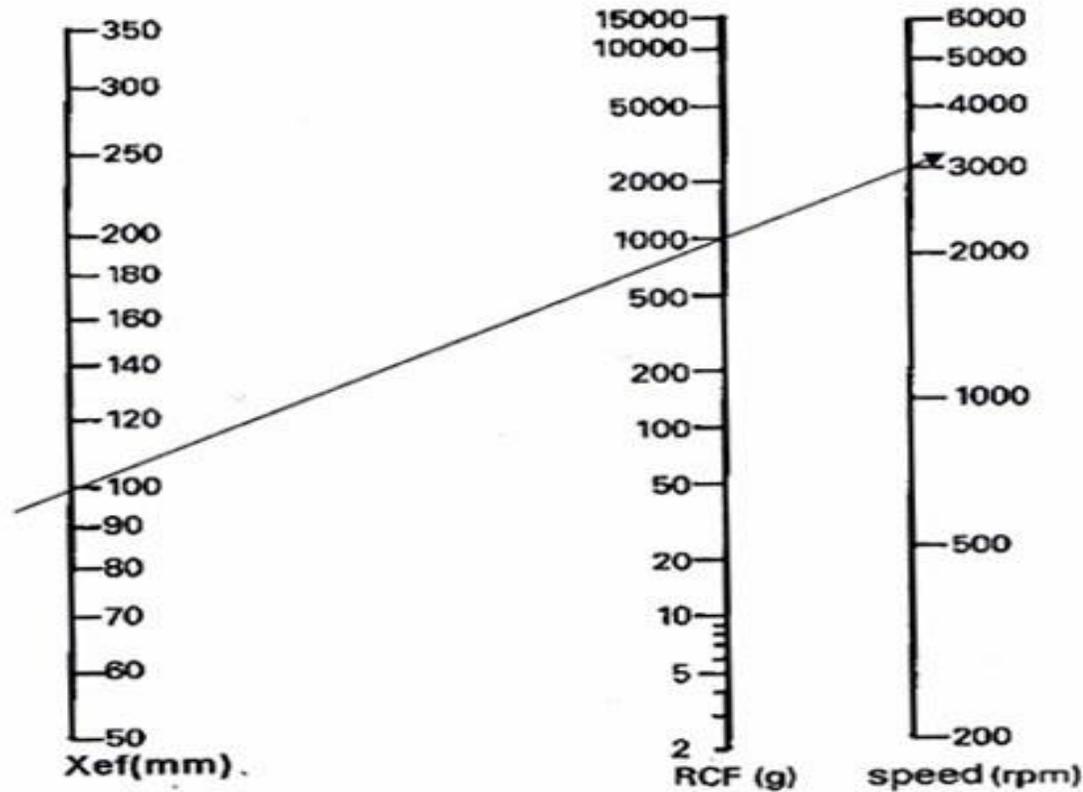
Example: 5000 RPM in a small centrifuge has a different RCF than in a large centrifuge.

This means that the RCF is always the same regardless of centrifuge size.

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How to determine RCF using RPM and the other way around?

Nomogram:

Falcon. (2013). *Nomograph for RCF Determination* *https://www.corning.com/catalog/cls/documents/application-notes/an_DL_084_Nomograph_for_RCF_Determination.pdf*

Formula: **RCF (relative centrifugal force) or G-force= 1.12 x r x (RPM/1000)²**
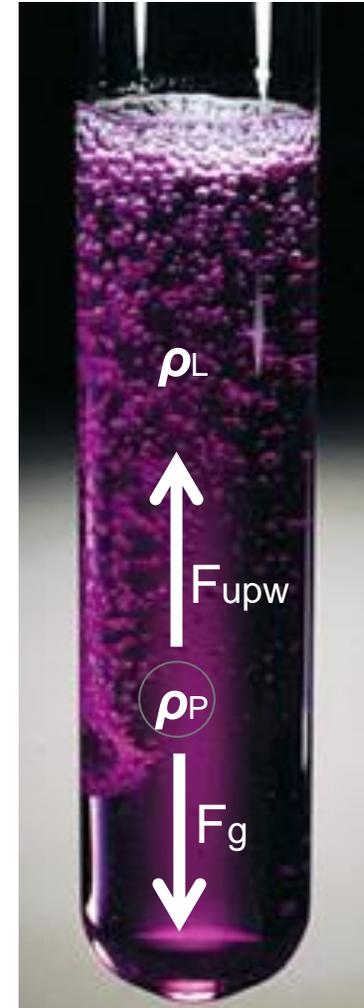
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Centrifugation: separate non-dissolved substances or particles

The sedimentation is dependent on:

- ✓ Size of particle
- ✓ Density of particle
- ✓ The viscosity of the solution /friction
- ✓ Speed/acceleration

ρL = Density Liquid
ρp = Density Particle
Fupw = Upward force
Fg = gravitational force
Ff = friction force

Forces on a particle in solution

➤ Gravitational force on every particle on earth:
F_g will depend on acceleration and Mass

$F_g = m \times g$

F in Newton = kg m s^{-2}
g = 9.81 m/s

➤ If the particle is in a solution:
F_{upw} upward force will arise

Fupw = Upward force
Fg = gravitational force

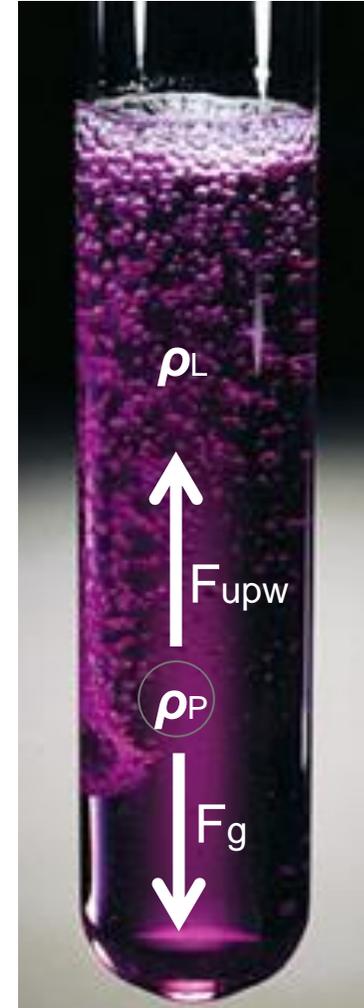# Forces on a particle in solution

$F_g = m_{particle\ p} \times g$

$F_{upw} = m_{displaced\ liquid\ (L)} \times g$

Mass (kg) = $\rho \cdot V$

$\rho = density\ (kg\ m^{-3})$
$V = volume\ (m^3)$

So: m_p depends on ρ_P and m_L depends on ρ_L

ρ_L

F_{upw}

ρ_P

F_g

Fg = gravitational force
Fupw = Upward force

ρL = Density Liquid
ρp = Density Particle

A particle in solution experiences friction ($F_{friction}$)

The friction depends on:
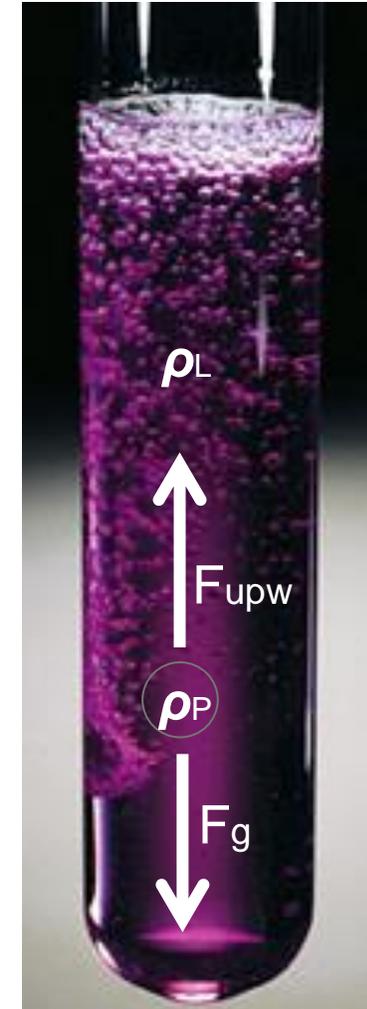- ✓ *Size of the particle*
- ✓ *Viscosity of the solution*

Friction force $F_f = f \cdot v$

For a spherical partical:
$f = 6 \cdot \pi \cdot \eta \cdot r_p$

So: Velocity $v = \dfrac{F_f}{f}$ $\rightarrow$ $v = \dfrac{F_f}{6\pi\eta r_p}$

f = friction coefficient
v = migration velocity
η = viscosity solution
r = radius particle

ρ_L

F_{upw}

ρ_P

F_g

Fg = gravitational force
Fupw = Upward force
ρL = Density Liquid
ρp = Density Particle

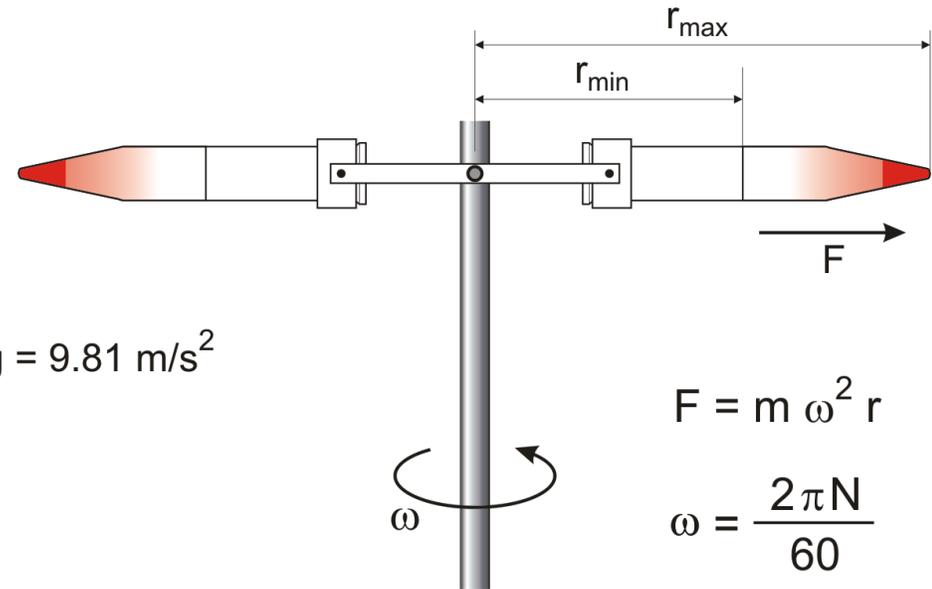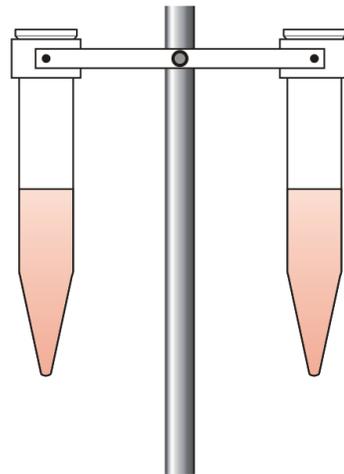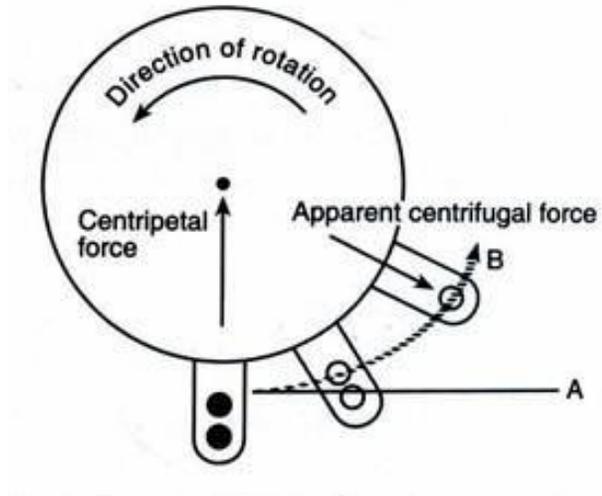# Sedimentation velocity without centrifugation (g = 9.81 m/s)

Particles (p)	Diameter (mm)	Sedimentation velocity
Blood cells	10	2 cm/hour
Cell nucleus	1	0,5 cm/day
Viruses	0,1	2 cm/year
Proteins	0,01	2 cm/century

So, to be able to seperate particles
we need to increase the sedimentation velocity

Centrifugation: increasing sedimentation velocity by increasing G-force

What is the influence of the radius (r) on the total force?

$$F = m\,\omega^2\,r$$

$$\omega = \frac{2\pi N}{60}$$

m = mass of particle

r = distance of particle from axis of rotation

ω = average angular velocity (rad/s)

N = revolution per minute, r.p.m.
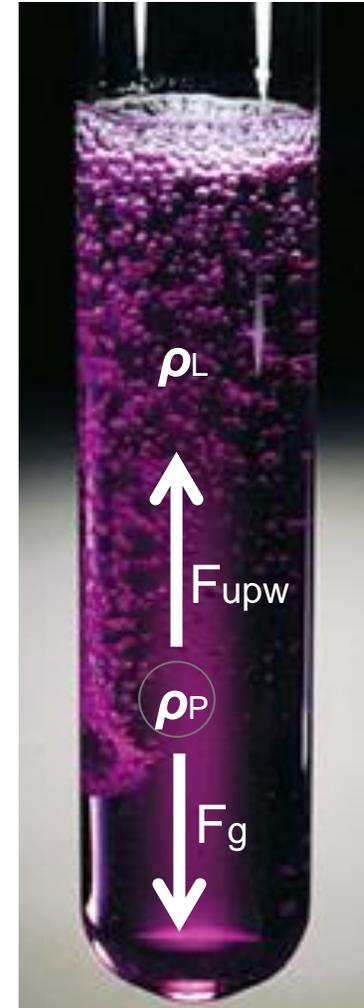
$g = 9.81 \text{ m/s}^2$

Centrifugation: increasing sedimentation velocity by increasing G-force

The sedimentation is dependent on:

- ✓ Size of particle
- ✓ Density of particle
- ✓ The viscosity of the solution /friction
- ✓ **Speed/acceleration**

ρL = Density Liquid
ρp = Density Particle
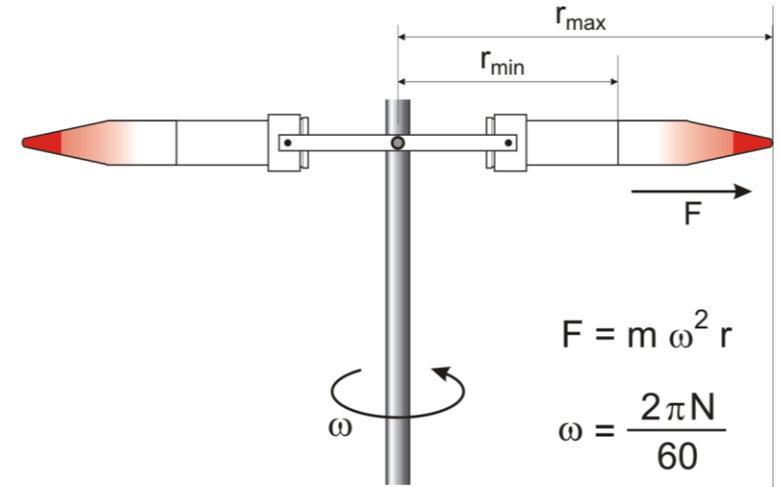Fupw = Upward force
Fg = gravitational force
Ff = friction force

Centrifugation: increase velocity by increasing acceleration

Sedimentation velocity v <=> a <=> G-force

Acceleration a = $\omega^2 \cdot r$

ω = angular velocity of particles: **$2\pi \cdot N / 60$**
r = radius of rotation in mm

*ω = velocity in radial/**sec**
N = rounds per **minute** (rpm)
60 seconds = 1 minute*

$$F = m\,\omega^2\,r$$

$$\omega = \frac{2\pi N}{60}$$

m = mass of particle
r = distance of particle from axis of rotation
ω = average angular velocity (rad/s)
N = revolution per minute, r.p.m.

r_{max}
r_{min}
F

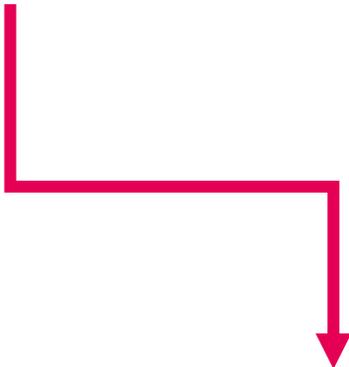# Centrifugation and forces: combining the formula's

$F_f = f \cdot v \quad \rightarrow \quad v = \dfrac{F_f}{f}$

$F = m \cdot g$

$F_f = \text{Particle - Liquid}$

$m = \rho \cdot V$

$f = 6 \cdot \pi \cdot \eta \cdot r_p$

$$v = \frac{F_f}{f} = \frac{(m_p - m_L) \cdot g}{6\pi \cdot \eta \cdot r_p} = \frac{(\rho_p - \rho_L) \cdot V \cdot g}{6\pi \cdot \eta \cdot r_p}$$

F_f = friction force (kg m s^{-2})
f = friction coefficient
v = velocity (m s^{-1})

m = mass (kg)
g = 9.81 m/s (constant)

η = viscosity solution
r_p = radius particle (mm)

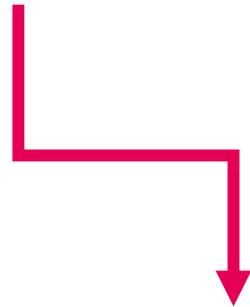ρ = density (kg m^{-3})
V = volume (m^3)

Centrifugation and forces: combining the formula's

$$v = \frac{(m_p - m_L) \cdot g}{6\pi \cdot \eta \cdot r_p}$$

Increase velocity by adding acceleration:

G-force = a = ω^2 . r

ω= 2π . N / 60 $\rightarrow$ a = $\left(\frac{2\pi \cdot N}{60}\right)^2$. r

$$v = \frac{(m_p - m_L) \cdot \left(\frac{2\pi \cdot N}{60}\right)^2 \cdot r}{6\pi \cdot \eta \cdot r_p}$$

F_f = friction force (kg m s^{-2})
f = friction coefficient
v= velocity (m s^{-1})

m = mass (kg)
g = 9.81 m/s (constant)

η = viscosity solution
r_p = radius particle (mm)

ρ = density (kg m^{-3})
V = volume (m^3)

a= acceleration (m s^{-2})
ω= angular velocity (rad s^{-1})
r = radius rotation (mm)
N = rounds per minute rpm

Centrifugation: a higher acceleration results in higher velocity

Sedementation velocity v <=> a <=> G-force

$$v = \{(m_P - m_L) \cdot (2\pi N/60)^2 \cdot r\}/ (6\pi\eta r_p)$$

Force on particle

Acceleration (N = rpm)

Distance to rotation axis

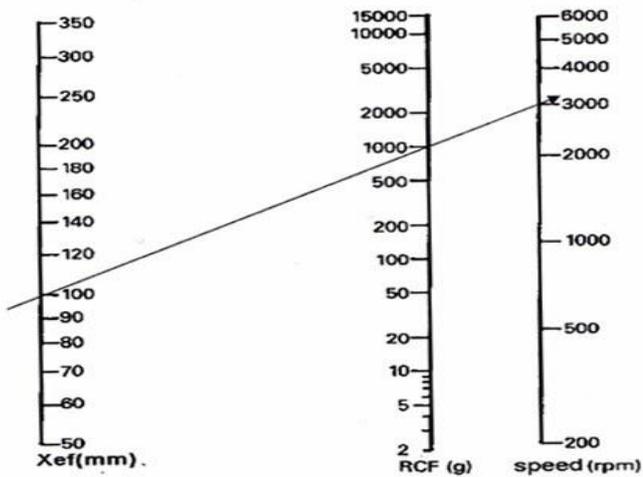*Sedimentation Coefficient*

This is also called *The effective rotor radius* x_{ef}

How are RCF and RMP related to each other?

RCF: The Relative Centrifugal Force
RPM: Revolutions Per Minute (RPM)

RCF or G-force= 1.12 x r x (RPM/1000)²

Centrifugation: How to go from RCF (g) to RPM (n)

RCF = a / 9.81

$RCF = Relative\ Centrifugational\ Force$
RCF = Relative Centrifugational Force
a = acceleration
9.81m/s = gravitational force constant

RCF = $(2\pi.N/60)^2$. r / 9.81

Replace 'N' with 'rpm'

$$RCF = \frac{\left(\frac{2\pi.N}{60}\right)^2.r}{9,81} = \frac{\left(\frac{2\pi.RPM}{60}\right)^2.r}{9,81}$$

1.12 = based on the constant gravitational force g (9.81 m/s) and π

rearrange

$$RCF = \frac{4\pi^2.RPM^2.r}{3600} . \frac{1}{9,81}$$

Simplify

RCF = $1.118. 10^{-5}$. r . RPM^2 **(r in cm)**

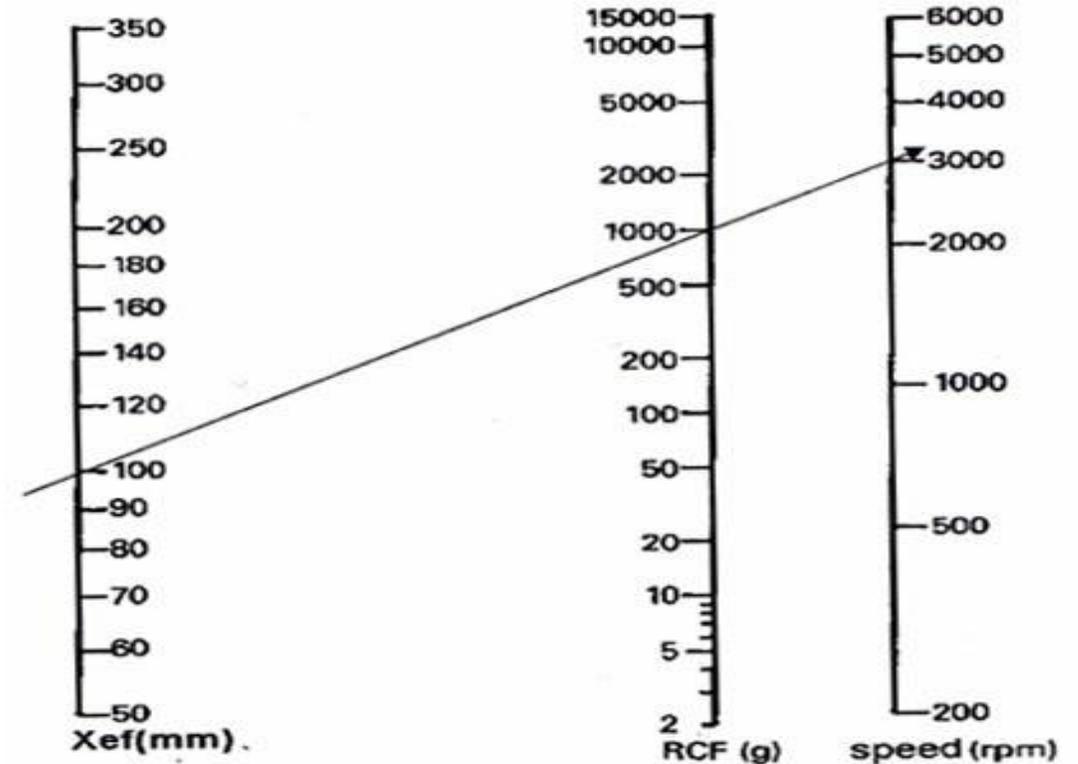**RCF** or G-force = 1.12 . r . $(RPM/1000)^2$ **(r in mm)**

Assignment 1: calculate from RMP to RCF

➤ **Use the formula below to proof that when rpm = 3000, RCF should indeed be around 1000**

RCF or G-force= 1.12 x r x (RPM/1000)²

Answer:
 - RCF or G-force= 1.12 x 100 x (3000/1000)²
 - RCF or G-force= 1.12 x 100 x 9 = 1008 g

Assignment 2: Is it possible to break a bacterial cell by centrifugation like a red blood cel?

- Hint 1: Check the size of a bacterial cell versus the size of eukaryotic cells

- Hint 2: $v = \dfrac{F_f}{f} = \dfrac{(m_p - m_L).g}{6\pi.\eta.r_p}$

Learning goals lesson 2: Centrifugation

After this lesson students:

- Can name different types of centrifugation

- Are able to explain the principle of centrifugation by explaining sedimentation, gravitational force, sedimentation velocity and acceleration.

- Know the difference between RCF and RPM, understand how the formula's are build up and are able to calculate from one to another.

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