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INTRUMENTASI BIOTEKNOLOGI

Program Studi Bioteknologi

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Pertemuan Ke 4

**Centrifuge,
Microcentrifuges, Vortex
mixer, Minispins, Sanicator
and Scale**



Centrifugation



Defenition

Centrifugation is a process that separates solids from liquids and liquids of different densities from each other by using centrifugal force

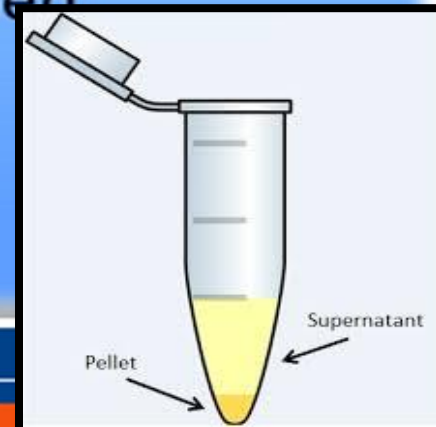
Another Defenition

- ✓ spins liquid samples at high speed.
- ✓ There are various types of centrifuges, depending on the size and the sample capacity.
- ✓ They vary widely in speed and capacity
- ✓ work by the sedimentation principle, where the centripetal acceleration is used to separate substances of greater and lesser density



PRINCIPLE

- The centrifuge works on the principle of increasing effective gravity to enhance the rate of settling of suspended particles more dense than the buffer.
- we need to double the force on samples, increase the rotation speed by 41%.
- After centrifuging, the liquid is called "supernatant" and the solids at the bottom of tube are called "pellet".



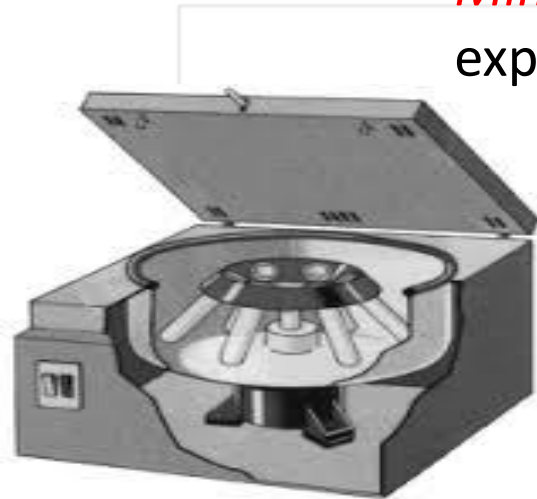
The rate of centrifugation is specified by the acceleration applied to the sample, typically measured in *Revolutions Per Minute* (RPM) or *Relative Centrifugal Force* (RCF). (RCF) expressed in units of gravity (times gravity or $\times g$).

The relationship between RPM and RCF is as follows

$$g = (1.118 \times 10^{-5}) R S^2$$



R is the radius of the rotor in centimeters,
S is the speed of the centrifuge in revolutions per minute (RPM)
g is values of RCF in units of times gravity



Speed (RPM)	Rotor Radius (from center of rotor to sample) in centimeters											
	4	5	6	7	8	9	10	11	12	13	14	15
1000	45	56	67	78	89	101	112	123	134	145	157	168
1500	101	126	151	176	201	226	252	277	302	327	352	377
2000	179	224	268	313	358	402	447	492	537	581	626	671
2500	280	349	419	489	559	629	699	769	839	908	978	1048
3000	402	503	604	704	805	906	1006	1107	1207	1308	1409	1509
3500	548	685	822	959	1096	1233	1370	1507	1643	1780	1917	2054
4000	716	894	1073	1252	1431	1610	1789	1968	2147	2325	2504	2683
4500	906	1132	1358	1585	1811	2038	2264	2490	2717	2943	3170	3396
5000	1118	1398	1677	1957	2236	2516	2795	3075	3354	3634	3913	4193
5500	1353	1691	2029	2367	2706	3044	3382	3720	4058	4397	4735	5073
6000	1610	2012	2415	2817	3220	3622	4025	4427	4830	5232	5635	6037
6500	1889	2362	2834	3306	3779	4251	4724	5196	5668	6141	6613	7085
7000	2191	2739	3287	3835	4383	4930	5478	6026	6574	7122	7669	8217
7500	2516	3144	3773	4402	5031	5660	6289	6918	7547	8175	8804	9433
8000	2862	3578	4293	5009	5724	6440	7155	7871	8586	9302	10017	10733
8500	3231	4039	4847	5654	6462	7270	8078	8885	9693	10501	11309	12116
9000	3622	4528	5433	6339	7245	8150	9056	9961	10867	11773	12678	13584
9500	4036	5045	6054	7063	8072	9081	10090	11099	12108	13117	14126	15135
10000	4472	5590	6708	7826	8944	10062	11180	12298	13416	14534	15652	16770
10500	4930	6163	7396	8628	9861	11093	12326	13559	14791	16024	17256	18489
11000	5411	6764	8117	9469	10822	12175	13528	14881	16233	17586	18939	20292
11500	5914	7393	8871	10350	11828	13307	14786	16264	17743	19221	20700	22178
12000	6440	8050	9660	11269	12879	14489	16099	17709	19319	20929	22539	24149
13000	7558	9447	11337	13226	15115	17005	18894	20784	22673	24562	26452	28341
13500	8150	10188	12225	14263	16300	18338	20376	22413	24451	26488	28526	30563
14000	8765	10956	13148	15339	17530	19722	21913	24104	26295	28487	30678	32869

Types of Centrifuges

1. Small Benchtop

- with or without refrigeration
- slow speed (eg up to 4000 RPM)
- common in clinical labs (blood/plasma/serum separation)
- can take approx (up to) 100 tubes, depending on diameter



2. Microcentrifuges (“microfuge”, “Eppendorf”)

- take tubes of small vols (up to 2 mL)
- very common in biochemistry/molecular biology/ biological labs
- can generate forces up to $\sim 15,000 \times g$
- with or without refrigeration



Types of Centrifuges

3. High Speed centrifuges

- 15,000 – 20,000 RPM
- large sample capacity depending on rotor
- normally refrigerated
- research applications



4. Ultracentrifuges → 65,000 RPM (100,000's x g)

- limited lifetime
- expensive
- require special rotors
- care in use – balance critical!
- research applications





Operation

The influence of the particles' settling velocity in centrifugation

- ✓ size and shape,
- ✓ centrifugal acceleration,
- ✓ the volume fraction of solids present,
- ✓ the density difference between the particle and the liquid
- ✓ viscosity of the medium
- ✓ rotor speed

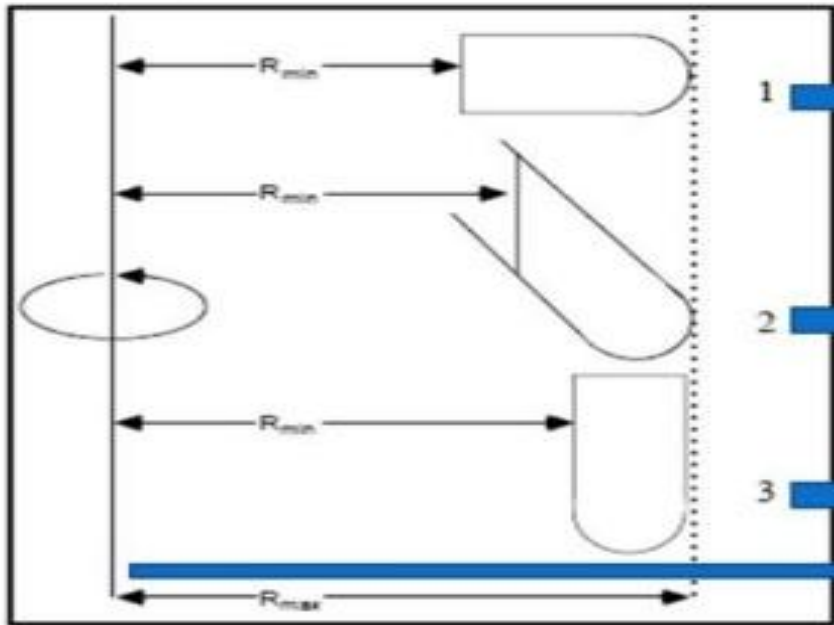
Centrifuge tubes

- **Glass centrifuge tubes** can be used with most solvents, but tend to be **more expensive**. They can be cleaned like other laboratory glassware, and can be **sterilized** by autoclaving.
- **Plastic centrifuge tubes**, especially micro-centrifuge tubes tend to be **less expensive**. Water is preferred when plastic centrifuge tubes are used. They are more difficult to clean thoroughly, and are usually inexpensive enough to be considered **disposable**



Centrifugal rotors

Types of rotors



1 → **Swinging bucket rotor**

2 → **Fixed angle rotor**

3 → **Vertical-tube rotor**

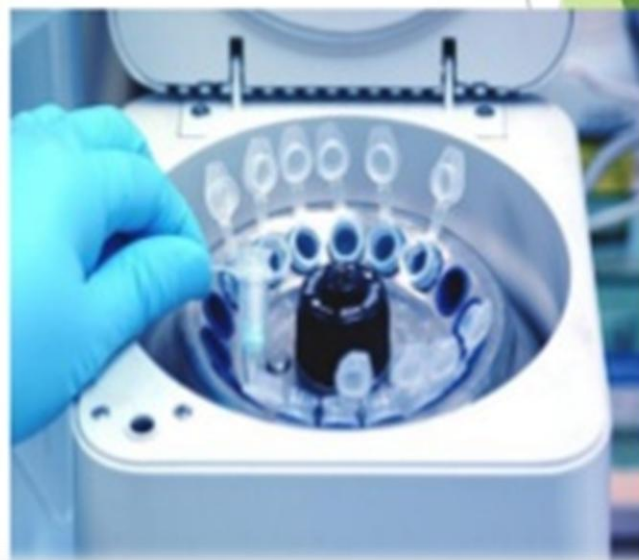
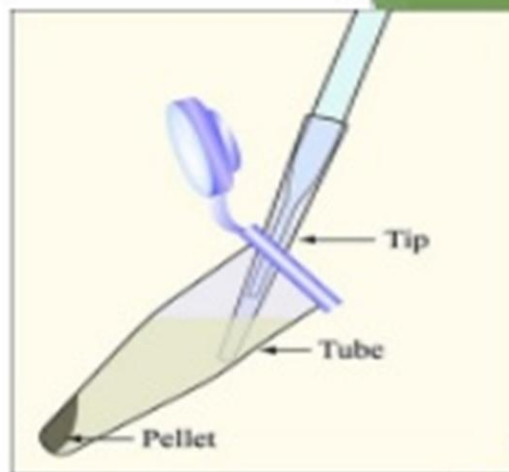
→ **Central shaft**

Fabrication: Carbon fiber, Aluminum and Titanium.



Micro centrifuge (“microfuge”, “Eppendorf”)

- Sample volume is small in eppendorf tubes
- Refrigerated with or without
- Centrifuge maximum approx 10000 g
- Take tube of small volume up to 2ml.
- Commonly used of concentration protein





Three microcentrifuge tubes: 2 mL, 1.5 mL and 200 µL (for [PCR](#)).

Care and Maintenance

- ✓ log book for centrifuge
- ✓ regular inspection by authorised personnel
- ✓ oiling, bearings, swivel points
- ✓ Cleanliness after used
- ✓ ENSURE TUBES ARE BALANCED (esp. ultracentrifuges)



Vortexs mixer



Defenition

A **vortex mixer**, or vortexer, is a simple device used commonly in laboratories to mix small vials of liquid. It consists of an [electric motor](#) with the drive shaft oriented vertically and attached to a cupped rubber piece mounted slightly off-center



Description of the unit

CONTINUOUS-SENSOR

- ✓ **Continuous** (indicator light off) allows continuous operation mode even with different accessories;
- ✓ **Sensor** (indicator light on) allows automatic operation mode even with different accessories.
- ✓ Speed Regulation knob of mixing speed from 0 to 3000 rpm

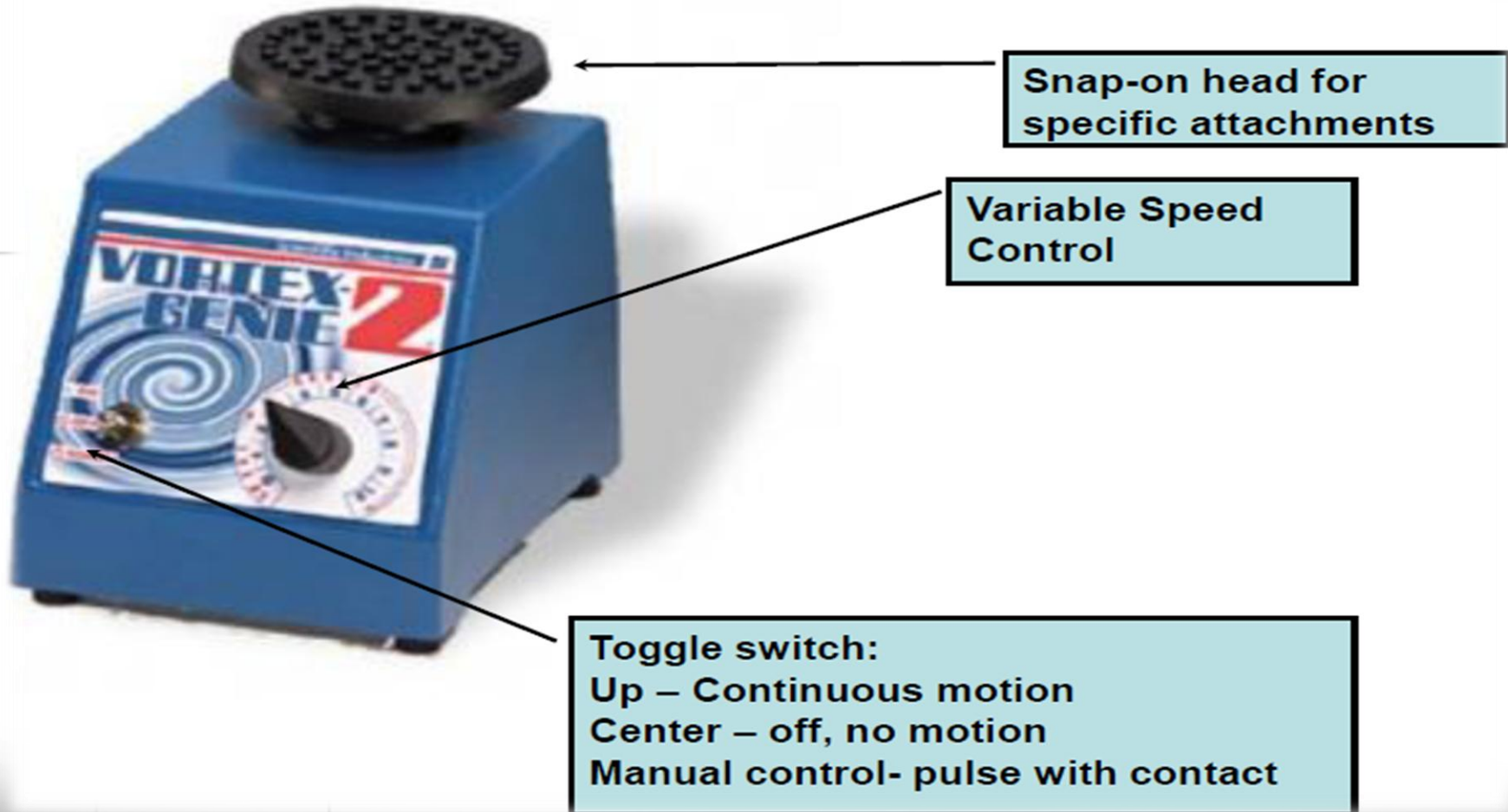
CONTINUOUS-TOUCH

respective modes:

- ✓ **Continuous** (indicator light off) allows continuous operation mode even with different accessories
- ✓ **Touch** (indicator light on) allows automatic operation mode even with different accessories



Part of Vortexs mixer





Minispin

NEW!
With PCR-
strip rotor

Defenition

Mini spin or spindown are bench-top “personal centrifuge” designed for workstations in training and research laboratories in fields of bioscience, medicine and chemistry

- ✓ spins liquid samples at high speed.
- ✓ microcentrifuge tube can be centrifuged
- ✓ fixed-angle rotor



Specifications:



- Capacity: 200 μ L - 2.0 mL (adapters available for smaller tubes)
- Maximum RCF: 12.000 $\times g$ (MiniSpin); 14.000 $\times g$ (MiniSpin Plus)
- Speed: 800-14,000rpm
- Acceleration time to max. speed: 13 sec
- Deceleration time from max. speed: 12 sec
- Clear, easy-to-use digital display of time and speed
- Remarkably quiet operation
- Metal rotor housing
- Separate "Short Spin" button for fast, convenient quick spins
- without refrigeration

Sanication



Defenition

Sonication : Is the act of applying sound energy or ultrasonic wave to agitate particles in a sample, for various purposes.

- Ultrasonic frequencies (>20 kHz) are usually used, leading to the process also being known as **ultrasonication**
- Sonication can be used to remove dissolved gases from liquids by sonicating the liquid

Application

Sonication can be used for the production of

- nanoparticles, such as [nanoemulsions](#), [nanocrystals](#), [liposomes](#)
- wax emulsions
- as well as for wastewater purification
- extraction of plant oil
- extraction of anthocyanins and antioxidants
- production of [biofuels](#)
- crude oil desulphurization, cell disruption polymer and epoxy processing, adhesive thinning, and many other processes

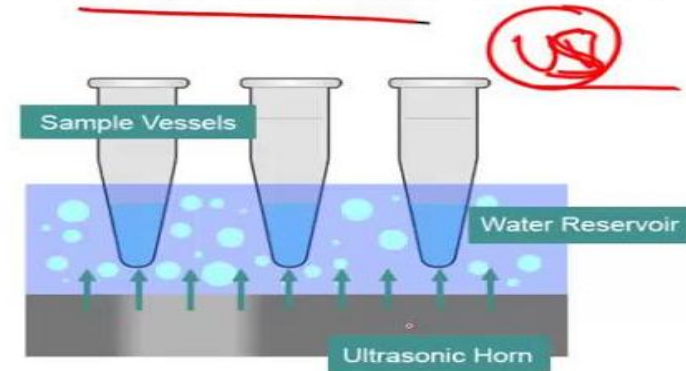
Types of Sonication

it is usually applied using an

- Sanicator ultrasonic bath



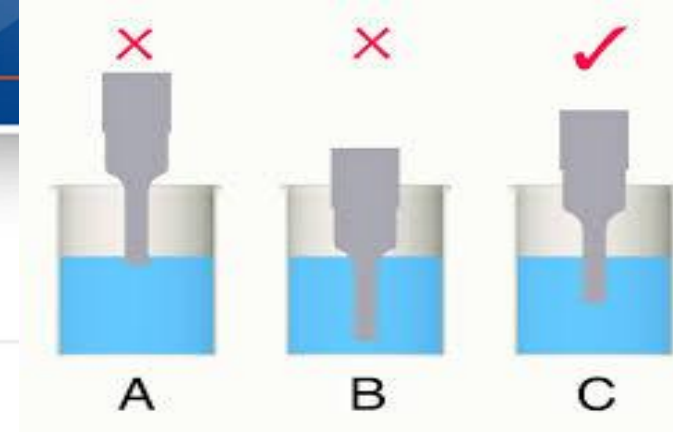
Sonication of cell



- non-conformable
- uncontrollably distributed through the tank.
- low intensity
- unevenly spread.
- The repeatability and scalability of the process is very poor

Types of Sonication

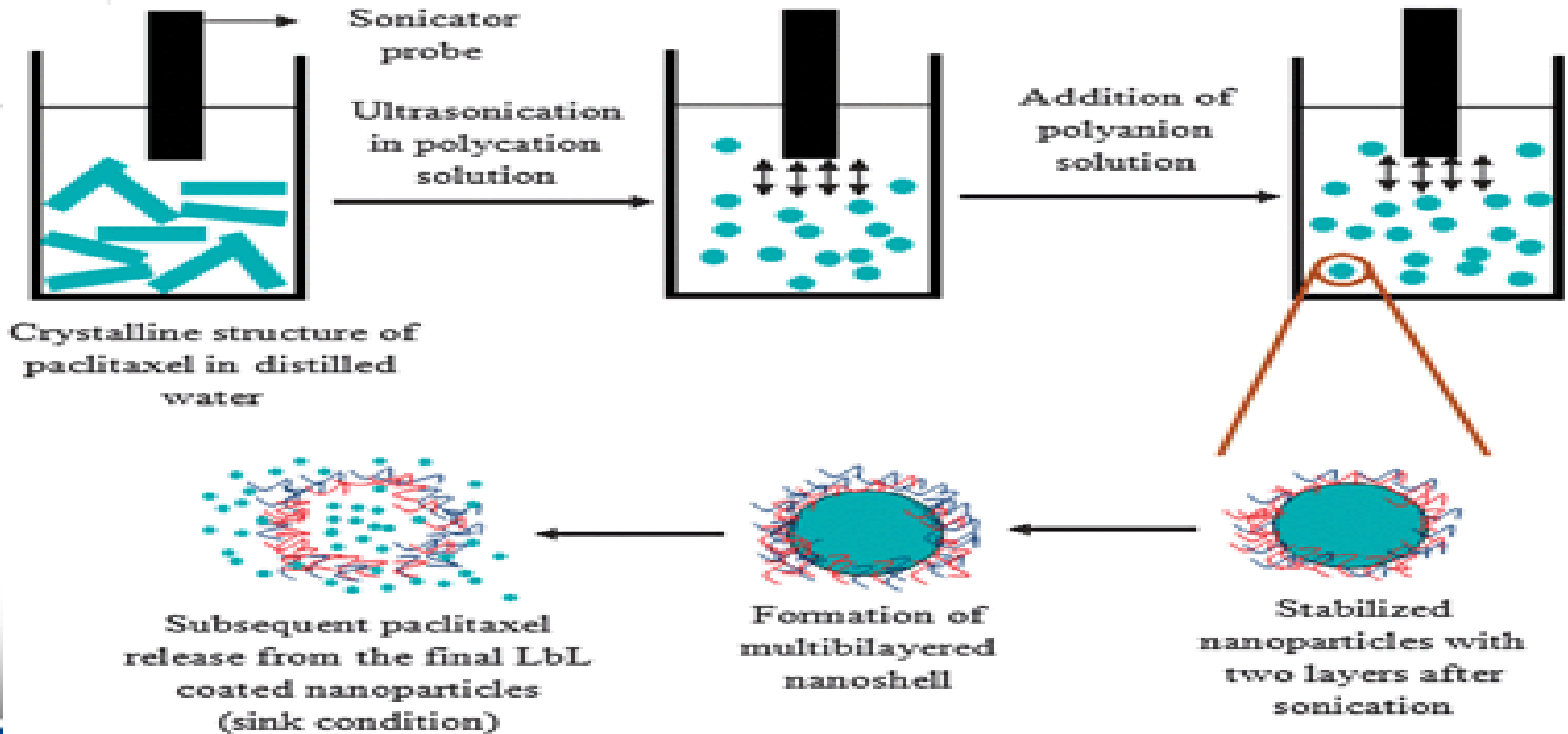
- Sanicator ultrasonic probe



- a higher intensity and efficiency of the sonication process
- full control over the most important parameters – amplitude, pressure, temperature, viscosity, concentration, reactor volume.

Types of Sonication

- Sanicator ultrasonic probe



Scale



Defenition

Measurement standard of mass and weight of an object with spesific accuracy established through calibration with respect to a primary measurement standard for a quantity of the same kind

The SI system of Units

- Kilogram
- Gram
- miligram



Mass Standards- Types of Masses



Masses made from brass and cast iron masses



Types and Classes of Balances

Weighing balances are classified into different types

The main categories are :

- Triple beam balances
- Two pan, three knife – edge balances
- Single pan, two knife – edge balances
- Analytical balance
- Mass comparator



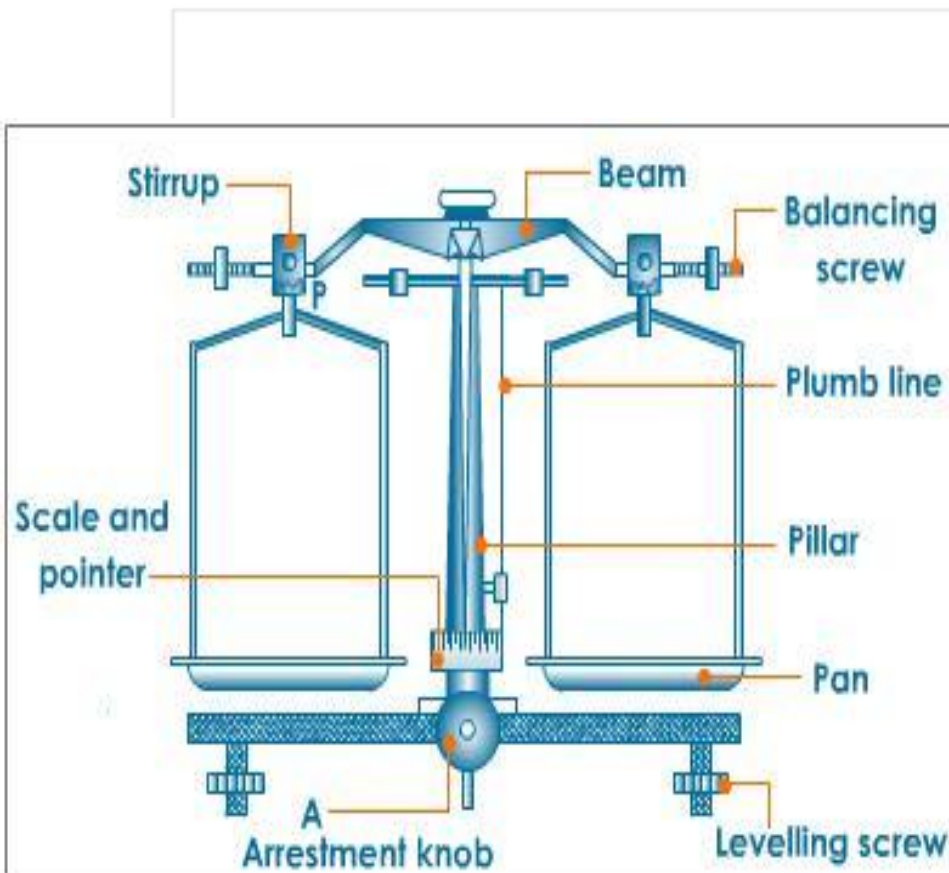
Triple beam balances



A triple beam balance is a type of balance commonly used in the laboratory to determine the mass (by weight comparison) of samples. It is called a triple beam because of the three beams on the scale that are used for determining the weight of the item. The first beam measures 0 to 10 grams, the middle beam weighs in 10 gram increments and the far beam weighs in 100 g increments.

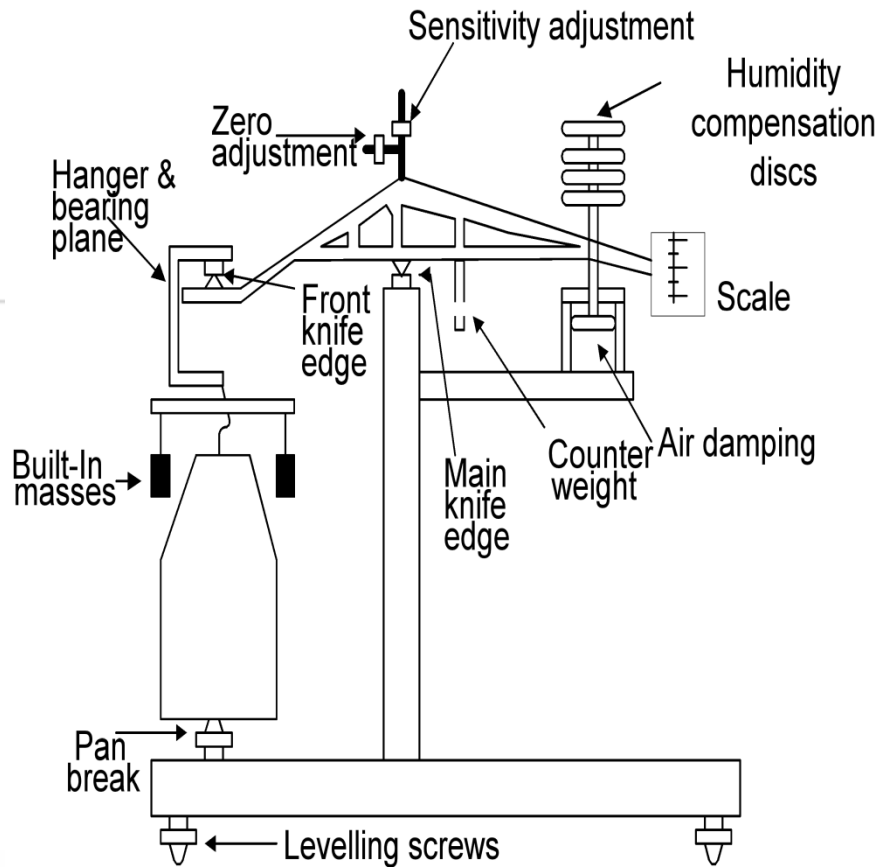


Two pan, three knife – edge balances



Two pan three knife balance consist of main beam carrying two pans at its ends. The central knife edge is normally equi-distant from the pan knife edges and due to this reason these balance are also known as equal arm balances. This type of balance is mostly used in high precision metrology laboratories for calibration of secondary level masses

Single pan, two knife – edge balances



single pan two knife balance consist of main beam carrying single pans at one ends. masses attached to the pan assembly so that whenever a load is placed on the pan an equivalent mass is lifted from the pan. This means that the load to be supported by the knife edges is fairly constant and balances of this type are often referred to as constant load balances.

Analytical balances



A class of balance designed to measure small mass in the sub-milligram range. The measuring pan of an analytical balance (0.1 mg or better) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation

Mass comperator



Mass comparators are used for comparison of precision masses. Generally the construction of these balances is similar to electromagnetic force compensation type but they are built with more precision and stability. Mass Comparators are available in capacities in the range $0.1 \mu\text{g}$ to 20 kg with excellent repeatability and linearity



Terima
kasih