# Methods of cell centrifugation

essay: Methods of Cell Centrifugation essay? O?°C? 4?°C: Cell organelles such as nuclei, mitochondria, etc., can be removed from the cell and separated from each other by centrifugation, which is done in a centrifuge Before centrifugation, cells are first disrupted either by grinding tissue in a mortar or homogenization in ground glass or exposure to sonic vibrations. In most cases, disruption is best done at low temperatures between O?°C and 4?°C, especially for the preservation of metabolic activity for subsequent study. After cell disruption, the particulate components are separated from each other by high speed centrifugation. There are two methods of centrifugation:(1) differential centrifugation and(2) gradient differential centrifugation. 1. Differential Centrifugation: It depends on differences in sedimentation rate among various components. The heavier particles settle first and then there is gradual separation by sedimentation of light particles. For example, centrifugation at a speed 700 times gravity of a tissue homogenate suspended in 0. 25M sucrose results in the separation of nuclei.

If the supernatant resulting from this centrifugation is centrifuged at about 5000 to 8500 times gravity, the mitochondria settle out of solution. At still higher speed, other particulate components are separated. For example, microsomal fraction in 0. 25M sucrose is obtained by 30 minute centrifugation at 100, 000 times gravity. 2. Gradient Differential Centrifugation: It depends on density gradient among different cellular components.

In this case the homogenate is placed in a tube on top of a sucrose column, which is stratified, i. e., sucrose solution progressively increases in density from top to bottom.

Upon centrifugation, cellular components having different sedimentation rates appear at different levels according to their size and specific gravity. For chemical components of cells, specific procedures are followed. For example, retention of nucleoprotein and enzymes of the nucleus, the tissue is freeze-dried (lyophilized) and suspended in a non-aqueous solvent (for nuclei, an ether-chloroform mixture or a benzene-carbon tetrachloride mixture). Cells are disrupted by grinding in a colloid mill or a mortar, and the nuclei are isolated by gradient differential centrifugation. essay? essay -X