Data Collection, Results and Presentation

Data Collection

The intensity of diffracted X-rays is continuously recorded as the sample and detector rotate through their respective angles. A peak in intensity occurs when the mineral contains lattice planes with d-spacings appropriate to diffract X-rays at that value of θ. Although each peak consists of two separate reflections ($K\alpha_1$ and $K\alpha_2$), at small values of 2θ the peak locations overlap with $K\alpha_2$ appearing as a hump on the side of $K\alpha_1$. Greater separation occurs at higher values of θ. Typically these combined peaks are treated as one. The 2λ position of the diffraction peak is typically measured as the center of the peak at 80% peak height.

Data Reduction

Results are commonly presented as peak positions at 2θ and X-ray counts (intensity) in the form of a table or an x-y plot (shown above). Intensity (*I*) is either reported as peak height intensity, that intensity above background, or as integrated intensity, the area under the peak. The relative intensity is recorded as the ratio of the peak intensity to that of the most intense peak (*relative intensity* = $I/I_1 \times 100$).

Determination of an Unknown

The d-spacing of each peak is then obtained by solution of the Bragg equation for the appropriate value of λ. Once all d-spacings have been determined, automated search/match routines compare the *d*s of the unknown to those of known materials. Because each mineral has a unique set of d-spacings, matching these d-spacings provides an identification of the unknown sample. A systematic procedure is used by ordering the d-spacings in terms of their intensity beginning with the most intense peak. Files of d-spacings for hundreds of thousands of inorganic compounds are available from the International Centre for Diffraction Data as the Powder Diffraction File (PDF). Many other sites contain d-spacings of minerals such as the American Mineralogist Crystal Structure Database. Commonly this information is an integral portion of the software that comes with the instrumentation.

Determination of Unit Cell Dimensions

For determination of unit cell parameters, each reflection must be indexed to a specific *hkl*.

Methods & Applications of Enzyme & Whole Cell Immobilization

Immobilization is defined as the imprisonment of cell or enzyme in a distinct support or matrix. The support or matrix on which the enzymes are immobilized allows the exchange of medium containing substrate or effector or inhibitor molecules. The practice of immobilization of cells is very old and the first immobilized enzyme was **amino acylase** of *Aspergillus oryzae* for the production of L-amino acids in Japan.

Advantages of immobilized enzymes:

(1). *Increased functional efficiency of enzyme*
(2). *Enhanced reproducibility of the process they are undertaking*
(3). *Reuse of enzyme*
(4). *Continuous use of enzyme*
(5). *Less labour input in the processes*
(6). *Saving in capital cost and investment of the process*
(7). *Minimum reaction time*
(8). *Less chance of contamination in products*
(9). *More stability of products*
(10). *Stable supply of products in the market*
(11). *Improved process control*
(12). *High enzyme substrate ratio*

Disadvantages of enzyme immobilization:

(1). Even though there are many advantages of immobilized enzymes, there are some disadvantages also.
(2). High cost for the isolation, purification and recovery of active enzyme (most important disadvantage)
(3). Industrial applications are limited and only very few industries are using immobilized enzymes or immobilized whole cells.
(4). Catalytic properties of some enzymes are reduced or completely lost after their immobilization on support or carrier.
(5). Some enzymes become unstable after immobilization.
(6). Enzymes are inactivated by the heat generated in the system

Applications of enzyme immobilization:

(1). Industrial production: Industrial production of antibiotics, beverages, amino acids etc. uses immobilized enzymes or whole cells.

(2). Biomedical applications: Immobilized enzymes are widely used in the diagnosis and treatment of many diseases. Immobilized enzymes can be used to overcome inborn metabolic disorders by the supply of immobilized enzymes. Immobilization techniques are effectively used in drug delivery systems especially to oncogenic sites.

(3). Food industry: Enzymes like pectinases and cellulases immobilized on suitable carriers are successfully used in the production of jams, jellies and syrups from fruits and vegetables.

(4). Research: A Research activity extensively uses many enzymes. The use of immobilized enzyme allow researcher to increase the efficiency of different enzymes such as Horse Radish Peroxidase (HRP) in blotting experiments and different Proteases for cell or organelle lysis.

(5). Production of bio-diesel from vegetable oils.

(6). Waste water management: treatment of sewage and industrial effluents.

(7). Textile industry: scouring, bio-polishing and desizing of fabrics.

(8). Detergent industry: immobilization of lipase enzyme for effective dirt removal from cloths.

Supports or Matrix used in immobilization technology:

The matrix or support immobilizes the enzyme by holding it permanently or temporarily for a brief period of time. There are a wide variety of matrixes or carriers or supports available for immobilization. The matrix used should be cheap and easily available. Their reaction with the components of the medium or with the enzyme should be minimum as possible. The matrixes or supports for immobilization of enzymes or whole cells are grouped into three major categories

(1). Natural polymers

(2). Synthetic polymers

(3). Inorganic materials

(1). Natural polymers:

(a). Alginate: A natural polymer derived from the cell wall of some algae. Calcium or magnesium alginate is the most commonly used matrix. They are inert and have good water holding capacity.

(b). Chitosan and chitin: They are structural polysaccharides occurring naturally in the cell wall of fungi and the exoskeleton of Arthropods. The various functional groups in enzymes can bind to the – OH group of chitin and can form covalent bonds.

(c). Collagen: It is the protenaceous support with good porosity and water holding capacity. The side chains of the amino acids in the collagen and that of enzyme can form covalent bonds to permanently hold the enzyme to the support.

(d). Carrageenan: It is a sulfated polysaccharide obtained from some red algae. Their good gelling properties together with its high protein holding capacity makes it good support for immobilizing enzymes.

(e). Gelatin: Gelatin is the partially hydrolyzed collagen with good water holding capacity.

(f). Cellulose: Most abundant polymer of nature and it is the cheapest support available as carrier of enzymes. The hydroxyl group of the monomer units (glucose) can form covalent bonds with that of the amino acids of enzyme.

(g). Starch: A natural polymer of amylose and amylo-pectin. It has good water holding capacity.

(h). Pectin: It is a structural polysaccharide of plants found in their primary cell wall and they also acts as the inter-cellular cementing material in plant tissues. Pectin is a gelling agent with good water holding capacity.

(2). Synthetic polymers:

They are ion exchange resins or polymers and are insoluble supports with porous surface. Their porous surface can trap and hold the enzymes or whole cells. Example: Diethylaminoethyl cellulose (DEAE cellulose), Polyvinyl chloride (PVC), UV activated Polyethylene glycol (PEG)

(3). Inorganic materials:

(a). Zeolites: They are microporous, aluminosilicate minerals with good adsorbing properties and extensively used for immobilizing enzymes and whole cells.

(b). Ceramics: They are nonmetallic solids consisting of metal and nonmetal atoms held in ionic and covalent bonds. The composition and bonding pattern varies with different types.

(c). Diatomaceous earth: They are silicious sedimentary rocks formed by fossilized accumulations of the cell wall of diatoms. Celite is the trade name of diatomaceous earth. It is a good adsorbent and are resistant to high pH and temperature.

(d). Silica:

(e). Glass:

(f). Activated carbon

(g). Charcoal

Methods of Immobilization:

Based on support or matrix and the type of bonds involved, there are five different methods of immobilization of enzyme or whole cells.

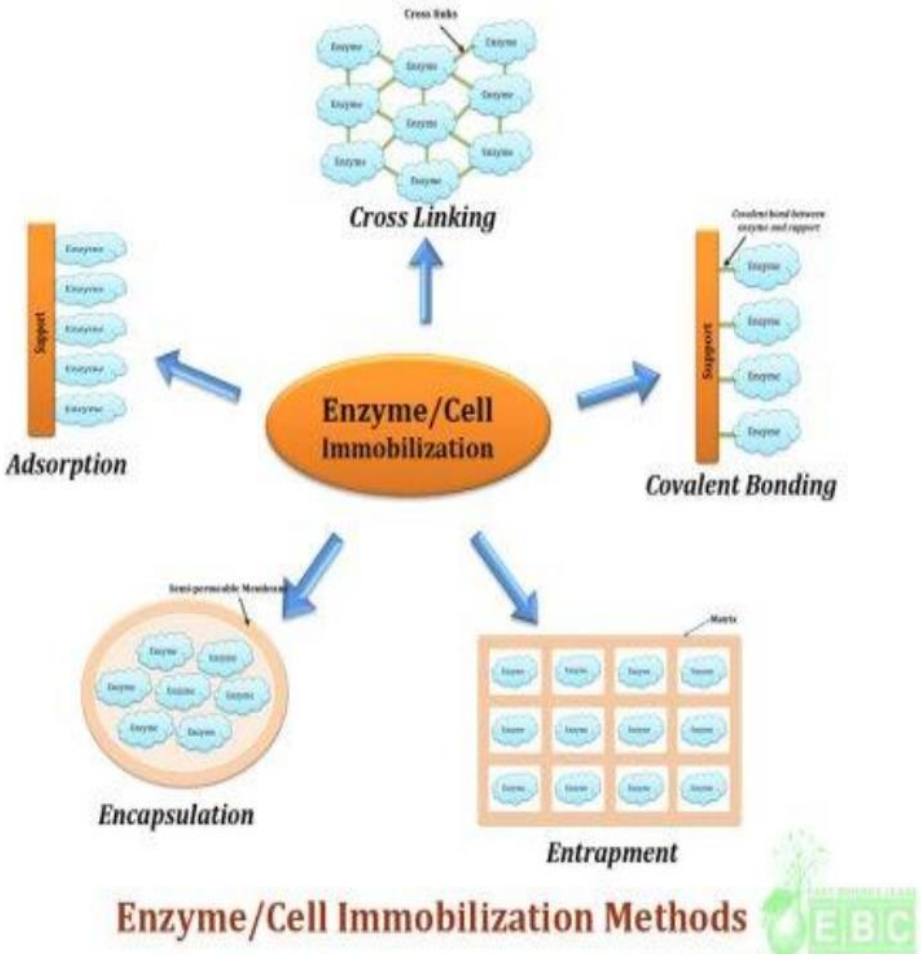
(1). Adsorption

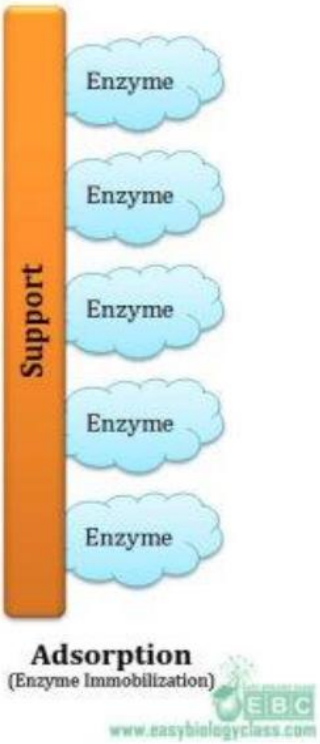
(2). Covalent bonding

(3). Entrapment

(4). Copolymerization

(5). Encapsulation





(1). Adsorption

Adsorption is the oldest and simplest method of enzyme immobilization. Nelson & Griffin used charcoal to adsorb invertase for the first time in 1916. In this method enzyme is adsorbed to external surface of the support. The support or carrier used may be of different types such as:

*(1). Mineral suppor*t (Eg. aluminum oxide, clay)

(2). Organic support (Eg. starch)

(3). Modified sepharose and ion exchange resins

There is no permanent bond formation between carrier and the enzyme in adsorption method. Only weak bonds stabilize the enzymes to the support or carrier. The weak bonds (low energy bonds) involved are mainly:

(a). Ionic interaction

(b). Hydrogen bonds

(c). Van der Waal forces

For significant surface bonding the carrier particle size must be small (500 Å to 1 mm diameter). The greatest advantage of adsorption method is that there will not be "pore diffusion limitations" since enzymes are immobilized externally on the support or the carrier.

Methods of adsorption:

(1). Static process: Immobilization to carrier by allowing the solution containing enzyme to contact the carrier without stirring.

(2). Dynamic batch process: Carrier is placed in the enzyme solution and mixed by stirring or agitation.

(3). Reactor loading process: Carrier is placed in the reactor, and then the enzyme solution is transferred to the reactor with continuous agitation.

(4). Electrode position process: Carrier is placed near to an electrode in an enzyme bath and then the current is put on, under the electric field the enzyme migrates to the carrier and deposited on its surface.

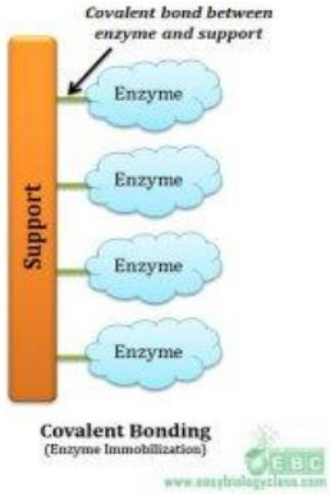
Advantages of adsorption method:

(a). No pore diffusion limitation
(b). Easy to carry out
(c). No reagents are required
(d). Minimum activation steps involved
(e). Comparatively cheap method of immobilization
(f). Less disruptive to enzyme than chemical methods

Disadvantages of adsorption method:

(a). Desorption of enzymes from the carrier
(b). Efficiency is less

(2). Covalent bonding:



This method involves the formation of covalent bonds between the chemical groups in enzyme and to the chemical groups on the support or carrier. It is one of the widely used methods of enzyme immobilization. Hydroxyl groups and amino groups of support or enzyme form covalent bonds more easily. Chemical groups in the support or carrier that can form covalent bonds with support are amino groups, imino groups, hydroxyl groups, carboxyl groups, thiol groups, methylthiol groups, guanidyl groups, imidazole groups and phenol ring.

Important functional groups of the enzyme that provide chemical groups to form covalent bonds with support or carrier are:

1. Alpha carboxyl group at 'C' terminal of enzyme
2. Alpha amino group at 'N' terminal of enzyme
3. Epsilon amino groups of Lysine and Arginine in the enzyme
4. β and γ carboxyl groups of Aspartate and Glutamate
5. Phenol ring of Tyrosine
6. Thiol group of Cysteine
7. Hydroxyl groups of Serine and Threonine
8. Imidazole group of Histidine
9. Indole ring of Tryptophan

Carriers or supports commonly used for covalent bonding are:

(a). Carbohydrates: Eg. Cellulose, DEAE cellulose, Agarose

(b). Synthetic agents: Eg. Polyacrylamide

(c). Protein carriers: Collagen, Gelatin

(d). Amino group bearing carriers: Eg. amino benzyl cellulose

(e). Inorganic carriers: Porous glass, silica

(f). Cyanogen bromide (CNBr)-agarose and CNBr Sepharose

Methods of covalent bonding

(1). Diazoation: Bonding between amino group of support and tyrosil or histidyl group of enzyme.

(2). Peptide bond: Bonding between amino or carboxyl groups of the support and that of the enzyme.

(3). Poly functional reagents: Use of a bi-functional or multifunctional reagent (glutaraldehyde) which forms covalent bonds between the amino group of the support and amino group of the enzyme.

Advantages of covalent bonding:

(a). Strong linkage of enzyme to the support

(b). No leakage or desorption problem

(c). Comparatively simple method

(d). A variety of support with different functional groups available

(e). Wide applicability

Disadvantages of covalent bonding (major problem with covalent bonding):

(a). Chemical modification of enzyme leading to the loss of functional conformation of enzyme.

(b). Enzyme inactivation by changes in the conformation when undergoes reactions at the active site. This can be overcome through immobilization in the presence of enzyme's substrate or a competitive inhibitor.

(3). Entrapment:

In this method enzymes are physically entrapped inside a porous matrix. Bonds involved in stabilizing the enzyme to the matrix may be covalent or non-covalent. The matrix used will

be a water soluble polymer. The form and nature of matrix varies with different enzymes. Pore size of matrix is adjusted to prevent the loss of enzyme. Pore size of the matrix can be adjusted with the concentration of the polymer used. Agar-agar and carrageenan have comparatively large pore sizes. The greatest disadvantage of this method is that there is a possibility of leakage of low molecular weight enzymes from the matrix.

Examples of commonly used matrixes for entrapment are:

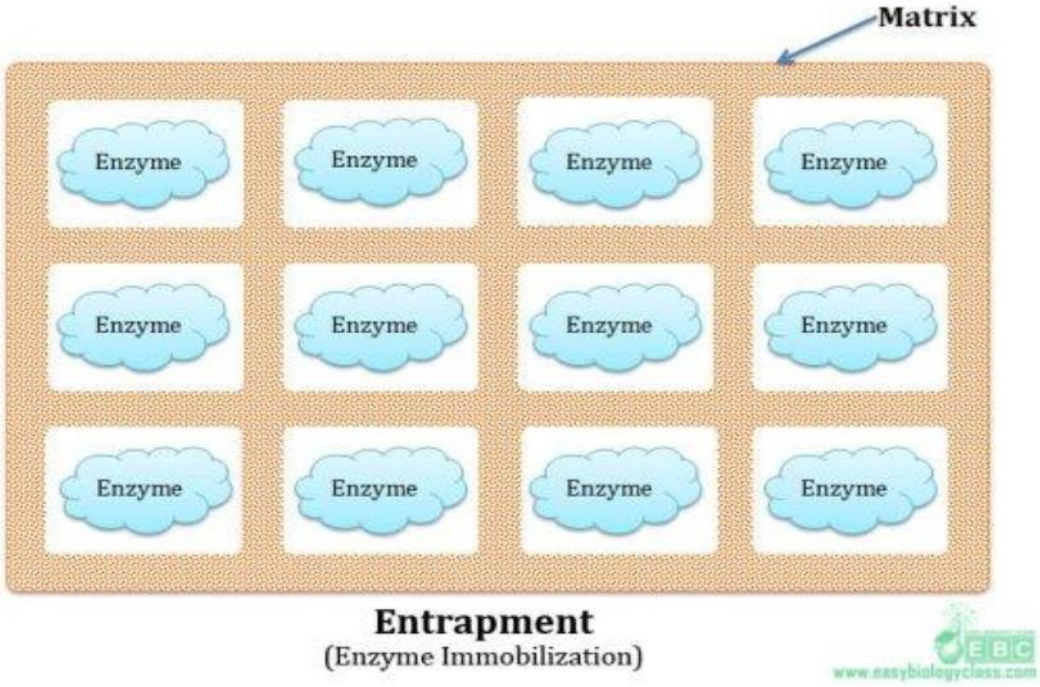
(1). Polyacrylamide gels
(2). Cellulose triacetate
(3). Agar
(4). Gelatin
(5). Carrageenan
(6). Alginate

Methods of entrapment:

(a). Inclusion in the gels: enzymes trapped inside the gels.

(b). Inclusion in fibers: enzymes supported on fibers made of matrix material.

(c). Inclusion in microcapsules: Enzymes entrapped in microcapsules formed by monomer mixtures such as polyamine and calcium alginate.



Advantages of entrapment:

(a). Fast method of immobilization

(b). Cheap (low cost matrixes available)

(c). Easy to practice at small scale

(d). Mild conditions are required

(e). Less chance of conformational changes in enzyme

(f). Can be used for sensing application

Disadvantages of entrapment:

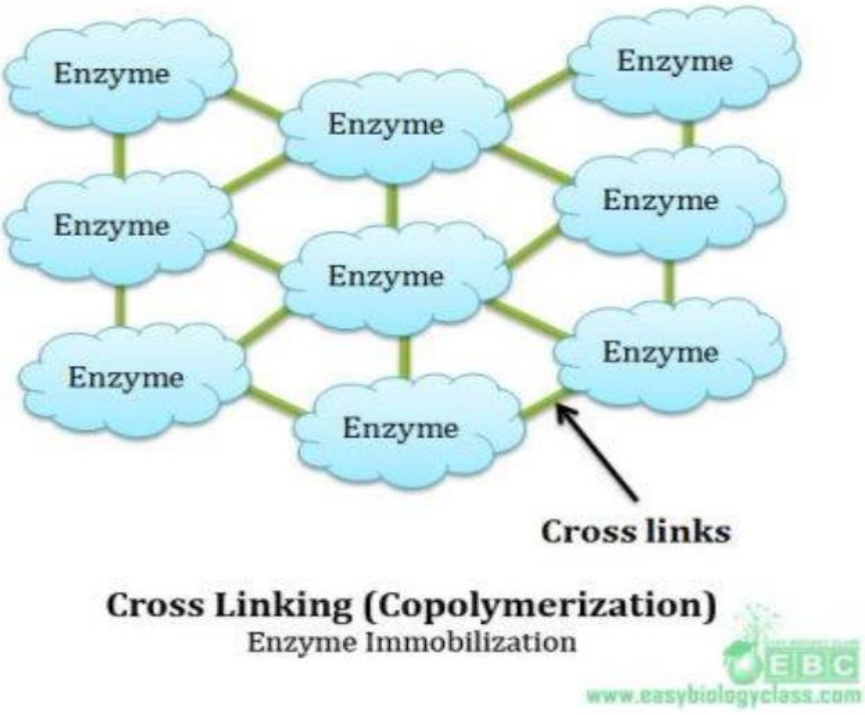
(a). Leakage of enzyme

(b). Pore diffusion limitation

(c). Chance of microbial contamination

(d). Not much success in industrial process

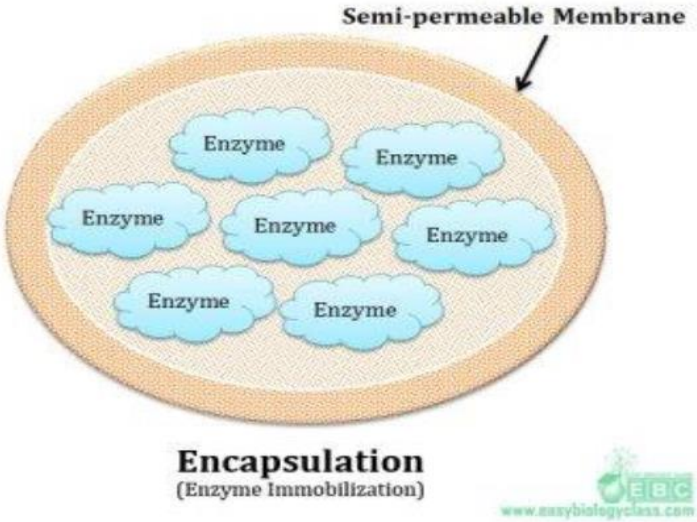
(4). Cross linking (copolymerization):



This method is also called as copolymerization. In this method of immobilization enzymes are directly linked by covalent bonds between various groups of enzymes via polyfunctional reagents. Unlike other methods, there is no matrix or support involved in this method. Commonly used polyfunctional reagents are glutaraldehyde and diazonium salt. This technique is cheap and simple but not often used with pure enzymes. This method is widely used in commercial preparations and industrial applications. The greatest disadvantage or demerit of this method is that the polyfunctional reagents used for cross linking the enzyme may denature or structurally modify the enzyme leading to the loss of catalytic properties.

(5). Encapsulation:

This type of immobilization is done by enclosing the enzymes in a membrane capsule. The capsule will be made up of semi permeable membrane like nitro cellulose or nylon. In this method the effectiveness depends upon the stability of enzymes inside the capsule.



Advantages of encapsulation:

(a). Cheap and simple method

(b). Large quantity of enzymes can be immobilized by encapsulation

Disadvantages of encapsulation:

(a). Pore size limitation

(c). Only small substrate molecule is able to cross the membrane

Immobilization of whole cells:

Immobilization of whole cells is an alternative to enzyme immobilization and it is a well-developed method for the utilization of enzymes from microbes. Immobilization of whole cells become particularly effective when the individual enzymes become inactive during direct immobilization, or the isolation and purification of enzyme is not cost effective. The greatest advantage of whole cell immobilization is that here the enzymes will be active and stable for long period of time since they are in their natural environment. Use of immobilized cells for fermentation is a very old practice. Bacteria or yeast cells are immobilized by adsorbing it on woodchips. This is practiced in many parts for different types of fermentations.

Advantages of whole cell immobilization:

(a). Multiple enzymes can be introduced to a single step

(b). Extraction and purification of enzymes are not required

(c). Enzymes are stable for long time

(d). Native conformation of enzyme is best maintained

(e). Cell organelles like mitochondria and chloroplasts can be immobilized

(f). Cost effective method

Disadvantages of whole cell immobilization:

(a). Concentration of enzymes will be less

(b). Production of unwanted enzymes and unwanted products

(c). Modification of end products by other enzymes produced by immobilized cells

Methods of immobilization of whole cells:

Methods of immobilization of whole cells are same as that described for enzyme immobilization and they include:

1. **Adsorption**
2. **Covalent bonding**
3. **Cell to cell cross linking**
4. **Encapsulation**
5. **Entrapment**

Immobilization of cells: Methods, Support materials, Cells and Reaction:

Method	Support Material	Cells	Reaction
Adsorption	Gelatin	*Lactobacilli*	Lactose ⟹ lactic acid
	Porous glass	*Saccharomyces*	Glucose ⟹ ethanol
	Cotton fibers	*Zymomonas*	Glucose ⟹ ethanol
	DEAE Cellulose	*Nocardia*	Steroid conversion
Covalent bonding	Cellulose + cyanuric chloride	*S. cerevisiae*	Glucose ⟹ ethanol
	Titanium oxide	*Acetobacter*	Vinegar
Cross linking	Glutaraldehyde	*E. coli*	Fumaric acid
Entrapment	Aluminium alginate	*Candida tropicalis*	Phenol degradation
	Calcium alginate	*S. cervisiae*	Glucose ⟹ ethanol
Encapsulation	Polyester	*Streptomyces sps.*	Glucose ⟹ fructose
	Alginate polylysine	Hybridoma cells	Monoclonal antibodies

Supercritical fluid extraction

Extraction can be defined as the removal of soluble material from an insoluble residue, either liquid or solid, by treatment with a liquid solvent. It is therefore, a solution process and depends on the mass transfer phenomena. The controlling factor in the rate of extraction is normally the rate of diffusion of the solute through the liquid boundary layer at the interface. Extraction involves the separation of medicinally active portions of plant or animal tissue from the inactive or inert components using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. So extraction continues to be of considerable interest in order to obtain improved yields of drug derived from plant and animal sources.

Techniques of extraction methods continue to be investigated and applied to obtain higher yields of the active substances from natural sources.

The principle methods of extraction are –

- Maceration
- Percolation
- Digestion

- Infusion
- Decoction

Solvent extraction

Solvent extraction also known as liquid-liquid extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. It is a basic technique in chemical laboratories, where it is performed using a separatory funnel. In other words, this is the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. Solvent extraction may be made use analytically for concentrating or rejecting a particular substance, or for the separation of mixtures. This process usually separates a soluble compound from an insoluble compound. Solvent extraction is used in nuclear processing, ore processing, production of fine organic compounds, processing of perfumes and other industries. Thus, although the method can be used for extraction and preconcentration of a wide range of non volatile or semi volatile species from water using only routine laboratory equipments, its use is decreasing in most situations because solvents of the required purity tend to be expensive, and can also cause problems with proper disposal after use.

Supercritical fluid extraction

Often the analysis of complex materials requires, as a preliminary step that is, separation of the analyte or analytes from a sample matrix. Ideally, an analytical separation method should be rapid, simple and inexpensive; should give quantitative recovery of analytes without loss or degradation; should yield a solution of the analyte that is sufficiently concentrated to permit the final measurement to be made without the need for concentration; should generate little or no laboratory wastes that have to be disposed off. For many years, one of the most common methods for performing analytical separations on complex environmental, pharmaceutical, food and petroleum samples was based upon extraction of bulk samples with hydrocarbon or chlorinated organic solvents using a Soxhlet extractor. Unfortunately, liquid extraction frequently fails to meet several of the ideal criteria. They usually require several hours or more to achieve satisfactory recoveries of analytes and even sometimes fails to do. The solvent costs are often high. The solutions of the recovered analytes are often so dilute so that a concentration step must follow the extraction. Analyte degradation or loss as well as atmospheric pollution may accompany this concentration step. Beginning in the mid-1980, chemists began to explore the use of supercritical fluids for the separation of analytes from

the matrix of many samples of interest to industry and governmental agencies because use of reagents of this type avoids many of the problems for organic liquid extractants.

What is Supercritical Fluid Extraction?

Supercritical fluids have been investigated since last century, with the strongest commercial interest initially focusing on the use of supercritical toluene in petroleum and shale oil refining during the 1970s. Supercritical water is also being investigated as a means of destroying toxic wastes, and as an unusual synthesis medium. The biggest interest for the last decade has been the applications of supercritical carbon dioxide, because it has a nearambient critical temperature (310C), thus biological materials can be processed at temperatures around 350C. The density of the supercritical CO2 at around 200 bar pressure is close to that of hexane, and the solvation characteristics are also similar to hexane; thus, it acts as a non-polar solvent. Around the supercritical region, CO2 can dissolve triglycerides at concentrations up to 1% mass. The major advantage is that a small reduction in temperature, or a slightly larger reduction in pressure, will result in almost the entire solute precipitating out as the supercritical conditions are changed or made sub critical. Supercritical fluids can produce a product with no solvent residues. Examples of pilot and production scale products include decaffeinated coffee, cholesterol-free butter, low-fat meat, evening primrose oil, squalene from shark liver oil, etc. The solvation characteristics of supercritical CO2 can be modified by the addition of an entrainer, such as ethanol, however some entrainer remains as a solvent residue in the product, negating some of the advantages of the "residue-free" extraction. Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent.

Extraction is usually from a solid matrix, but it can also be from liquids. SFE can be used asa sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). Carbon dioxide (CO2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical CO2 are above the critical temperature of 31°C and critical pressure of 74 bar. Addition of modifiers may slightly alter this. Supercritical extraction mostly uses carbon dioxide at high pressure to extract the high value products from natural materials. Unlike other processes, the extraction process leaves no solvent residue behind. Moreover the CO2 is non-toxic, nonflammable, odorless, tasteless, inert, and inexpensive. Due to its low critical temperature 31°C, carbon dioxide is known to be perfectly adapted in food, aromas, essential oils and nutraceutical

industries. Supercritical fluid A supercritical fluid is any substance at a temperature and pressure above its critical point. It can diffuse through solids like a gas, and dissolve materials like a liquid. Additionally, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties of a supercritical fluid to be "fine-tuned". Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Carbon dioxide and water are the most commonly used supercritical fluids, being used for decaffeination and power generation, respectively. CO2 is the kind of extraction solvents for botanicals. It leaves no toxic residue behind. Its extraction properties can be widely and precisely manipulated with subtle changes in pressure and temperature.

There are 2 types of CO2 extraction –

Low pressure cold extraction

It involves chilling CO2 at 35-550 F and pumping it through the plant material at between 800-1500 psi.

Supercritical extraction

It involves heating the CO2 to above 870 F and pumping it above 1100 psi. Usually, this is between 6000-10000 psi. Supercritical fluid CO2 can best be described as a dense fog when CO2 is used in a dense liquid state. Low-pressure CO2 is often the best method for producing high quality botanical extracts. CO2 loading rate in this state means that you have to pump many volumes of CO2 through botanical. The loading rate is typically 10-40 volumes. For this reason, it is important to have pumped CO2, which has a much faster loading rate 2-10 volumes and a wide range of uses.

Properties of supercritical fluid

(i) Supercritical fluids have highly compressed gases, which combine properties of gases and liquids in an intriguing manner.

(ii) Supercritical fluids can lead to reactions, which are difficult or even impossible to achieve in conventional solvents.

(iii) Supercritical fluids have solvent power similar to light hydrocarbons for most of the solutes. However, fluorinated compounds are often more soluble in supercritical CO2 than in hydrocarbons; this increased solubility is important for polymerization.

(iv) Solubility increases with increasing density (that is with increasing pressure). Rapid expansion of supercritical solutions leads to precipitation of a finely divided solid. This is a key feature of flow reactors.

(v) The fluids are commonly miscible with permanent gases (e.g. N2 or H2) and this leads to much higher concentrations of dissolved gases than can be achieved in conventional solvents.

Simple model of SFE

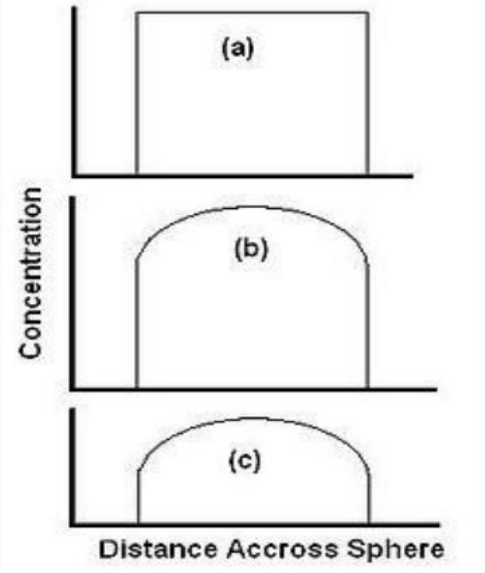


Figure 2. Concentration profiles during a typical SFE extraction

There are two essential steps to SFE, transport (by diffusion or otherwise) from with the solid particles to the surface, and dissolution in the supercritical fluid. Other factors, such as diffusion into the particle by the SF and reversible release such as desorption from an active site are sometimes significant, but not dealt with in detail here. Figure 2 shows the stages during extraction from a spherical particle where at the start of the extraction the level of extractant is equal across the whole sphere (Fig. 2a). As extraction commences, material is initially extracted from the edge of the sphere, and the concentration in the center is unchanged (Fig 2b). As the extraction progresses, the concentration in the center of the sphere drops as the extractant diffuses towards the edge of the sphere (Figure 2c).[6]

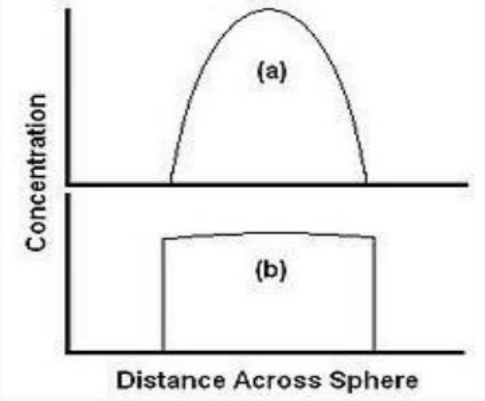


Figure 3. Concentration profiles for (a) diffusion limited and (b) solubility limited extraction

The relative rates of diffusion and dissolution are illustrated by two extreme cases in Figure 3. Figure 3a shows a case where dissolution is fast relative to diffusion. The material is carried away from the edge faster than it can diffuse from the center, so the concentration at the edge drops to zero. The material is carried away as fast as it arrives at the surface, and the extraction is completely diffusion limited. Here the rate of extraction can be increased by increasing diffusion rate, for example raising the temperature, but not by increasing the flow rate of the solvent. Figure 3b shows a case where solubility is low relative to diffusion. The extractant is able to diffuse to the edge faster than it can be carried away by the solvent, and the concentration profile is flat. In this case, the extraction rate can be increased by increasing the rate of dissolution, for example by increasing flow rate of the solvent.

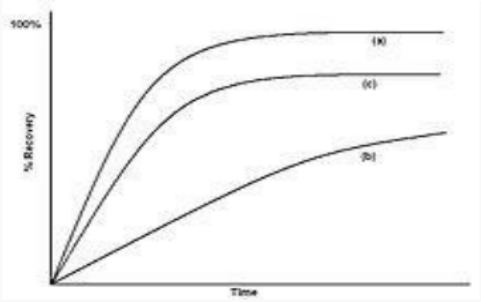


Figure 4. Extraction Profile for Different Types of Extraction

The extraction curve of % recovery against time can be used to elucidate the type of extraction occurring. Figure 4(a) shows a typical diffusion controlled curve. The extraction is initially rapid, until the concentration at the surface drops to zero, and the rate then becomes much slower. The % extracted eventually approaches 100%. Figure 4(b) shows a curve for a solubility limited extraction. The extraction rate is almost constant, and only flattens off towards the end of the extraction. Figure 4(c) shows a curve where there are significant matrix effects, where there is some sort of reversible interaction with the matrix, such as desorption from an active site. The recovery flattens off, and if the 100% value is not known, then it is hard to tell that extraction is less than complete.

Optimization

The optimum will depend on the purpose of the extraction. For an analytical extraction to determine, say, antioxidant content of a polymer, then the essential factors are complete extraction in the shortest time. However, for production of an essential oil extract from a plant, then quantity of CO_2 used will be a significant cost, and "complete" extraction not required, a yield of 70 - 80% perhaps being sufficient to provide economic returns. In another case, the selectivity may be more important, and a reduced rate of extraction will be

preferable if it provides greater discrimination. Therefore few comments can be made which are universally applicable. However, some general principles are outlined below.

Maximizing diffusion

This can be achieved by increasing the temperature, swelling the matrix, or reducing the particle size. Matrix swelling can sometimes be increased by increasing the pressure of the solvent, and by adding modifiers to the solvent. Some polymers and elastomers in particular are swelled dramatically by CO_2, with diffusion being increased by several orders of magnitude in some cases.

Maximizing solubility

Generally, higher pressure will increase solubility. The effect of temperature is less certain, as close to the critical point, increasing the temperature causes decreases in density, and hence dissolving power. At pressures well above the critical pressure, solubility is likely to increase with temperature.[8] Addition of low levels of modifiers (sometimes called entrainers), such as methanol and ethanol, can also significantly increase solubility, particularly of more polar compounds.

Optimizing flow rate

The flow rate of Supercritical carbon dioxide should be measured in terms of mass flow rather than by volume because the density of the CO_2 changes according to the temperature both before entering the pump heads and during compression. Coriolis flow meters are best used to achieve such flow confirmation. To maximize the rate of extraction, the flow rate should be high enough for the extraction to be completely diffusion limited (but this will be very wasteful of solvent). However, to minimize the amount of solvent used, the extraction should be completely solubility limited (which will take a very long time). Flow rate must therefore be determined depending on the competing factors of time and solvent costs, and also capital costs of pumps, heaters and heat exchangers. The optimum flow rate will probably be somewhere in the region where both solubility and diffusion are significant factors

Nanobiotechnology in molecular diagnostics

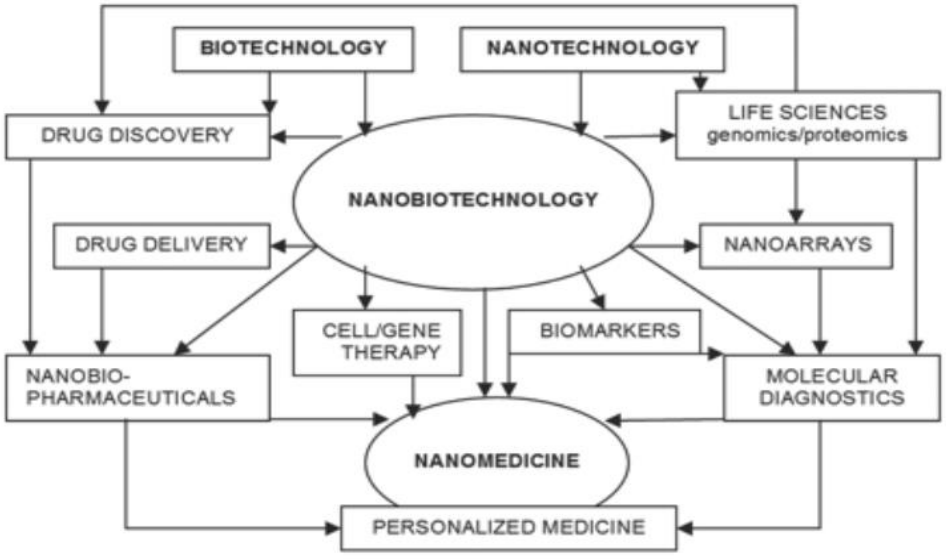
Nanomolecular diagnostics, the use of nanobiotechnology in molecular diagnostics, and nanobiotechnology, the use of various nanotechnologies and their applications in life sciences offer new options for clinical diagnostic procedures. No recognized classification

system for nanodiagnostics is currently in use, but a proposed system with the main categories of technologies is shown in Table 1.

Classification of categories of nanodiagnostic technologies.

Nanoscale visualization, e.g., atomic force microscopy, scanning probe microscopy
Nanoparticle biolabels
Nanotechnology-based biochips/microarrays
Nanoparticle-based nucleic acid diagnostics
Nanoproteomic-based diagnostics
Biobarcode assays
Nanopore technology
DNA nanomachines for molecular diagnostics
Nanoparticle-based immunoassays
Nanobiosensors
Combinations of multiple diagnostic technologies

Molecular diagnostics is an essential part of the development of personalized medicine, which features point-of-care performance of diagnostic procedures. Interrelationships of nanotechnology and molecular diagnostics and their role in nanomedicine as well as personalized medicine are shown schematically in Fig. 1



Nanoscale probes are suitable for detailed analysis of receptors, pores, and other components of living cells that are on a nanoscale. Thus nanotechnology can be used to improve PCR as well as provide non-PCR methods for rapid diagnostics. Advantages of applying nanotechnology to molecular diagnostics are that only small amounts of sample material are needed and that diagnostic tests that use nanoscale particles as tags or labels are faster and more sensitive (4). Nanoparticles can also be used to combine diagnostics with therapeutics

Nanotechnology-Based Biochips and Microarrays

Nanotechnology on a chip is a new paradigm for total chemical analysis systems (5). The ability to make chemical and biological information easier and less costly to obtain will impact molecular diagnostics and healthcare. Some examples of devices that incorporate nanotechnology-based biochips and microarrays are nanofluidic arrays and protein nanobiochips. These devices can be adapted for point-of-care use.

One of the more promising uses of nanofluidic devices is isolation and analysis of individual biomolecules, such as DNA. This capability could lead to new detection schemes for cancer. One such device entails the construction of silicon nanowires on a substrate, or chip, using standard photolithographic and etching techniques, followed by a chemical oxidation step that converts the nanowires into hollow nanotubes (6). With this process, the investigators can reliably create nanotubes with diameters as small as 10 nm, although devices used for biomolecule isolation contain nanotubes with diameters of 50 nm. Trapping DNA molecules requires a device consisting of a silicon nanotube connecting 2 parallel microfluidic channels. Electrodes provide a source of current used to drive DNA into the nanotubes. Each time a single DNA molecule moves into the nanotube, the electrical current suddenly changes. The current returns to its baseline value when the DNA molecule exits the nanotube. Nanofluidic technology is expected to have broad applications in systems biology, personalized medicine, pathogen detection, drug development, and clinical research.

Protein microarrays for the study of protein function are not widely used, in part because of the challenges in producing proteins to spot on the arrays. Protein microarrays can be generated by printing complementary DNAs onto glass slides and then translating target proteins with mammalian reticulocyte lysate (7). Epitope tags fused to the proteins allow them to be immobilized in situ. This procedure obviates the need to purify proteins, avoids protein stability problems during storage, and captures sufficient protein for functional studies. This technology has been used to map pairwise interactions among 29 human DNA

replication initiation proteins and to recapitulate the regulation of Cdt1 binding to select replication proteins and map the geminin-binding domain.