Optically active pharmaceutical compounds biology essay

The molecules which are non super imposable mirror images of one another are termed as chiral. These are a pair of enantiomers and are diasymmetric as well as optically active. Since they promote optical rotation, these enantiomers are also known as optical isomers. These chiral molecules consist of a tetrahedral carbon atom which is attached to four different groups. The carbon atom is the stereogenic or the asymmetric centre of the molecule. The enantiomers are similar in their physical and chemical properties in an achiral environment.

Enantiomers have different biological properties. This influences the efficacy and the toxicity of the compounds. Usually, one of the enantiomers is bioactive and the others may be inactive or toxic. Example, Verapamil is a calcium channel blocker used for the treatment for blood pressure, angina. The (S) isomer treats the increase in BP more effectively than the racemate form. The (R) isoform inhibits resistance of cancer cells to anti cancer drugs (Crosby, 1991).

The enantiomerically pure compounds are very useful and vital in the pharmaceutical and agrochemical industries. It has also been shown that the optically pure and chiral compounds should be used rather than mixture of enantiomers. The optically active pure compounds are used to produce antibodies, hormones, anti inflammatory, amino acids, vitamins, anti cancer drugs, cardiovascular drugs.

Chiral chromatography or ligand exchange chromatography was an analytical technique used for separating enantiomers. High performance liquid chromatography whereby chiral stationary phase is used was efficient

in separation of enantiomers. The optically active ligands like amino acids are bound covalently to a solid support, thereby forming a chiral stationary phase. Various amino acid derivatives like N -(3, 5-dinitrobenzoyl) phenyl glycines are also used.(Pirkle and Pochapsky, 1987). The major advantage of chromatography is that it results in high enantiomeric excess and is suitable on the analytical scale. However, its drawback is that the scale up is difficult.

The production of enantiomers for optically active drugs may be produced by different methods. Pure compounds are recovered by various extraction techniques from chiral compounds (alkaloids, carbohydrates) exist as pure enantiomers naturally. Fermentation of cheap substrates which are available in abundance (like molasses and sucrose) was a widely used source of single chiral molecules – lactic, tartaric and L- amino acids and also for complex substances which include vitamins, antibiotics and hormones.(Buchta, 1983).

Optically pure compounds may be prepared from inactive starting materials by asymmetric synthesis and resolution of racemates. In the process of asymmetric synthesis (Stinson, 1993) an enantiomeric reagent or catalyst is used for carrying out a specific reaction on an achiral substrate (prochiral) to produce a single chiral product. Overall, it is a selective technique as it leads to product selectivity. Its disadvantages are that it may be expensive due to the numerous steps involved and also because of the use of costly enantiomeric reagents. It is cheaper to produce a racemic mixture and then separate the enantiomers by physical methods like kinetic resolution or diastereomeric crystallization. Covalent derivatives are formed using optically pure resolving agents in the diastereomic crystallization method. The drawback is that it is wasteful since the unwanted isomer may be

discarded. On the other hand, kinetic resolution is based on the principle that two enantiomers react at variable rates in the presence of a chiral catalyst like an enzyme. This method involves product selectivity.

Biotransformation has also become a key technology used to produce chiral substances. It was used by many companies Eg. Celgene Corporation developed procedures to produce amines by using amino transferase (Celgene corporation, 1990). The main advantage of this procedure is that it allows 100% theoretical conversion of the substrate into the final product.

Membrane chirotechnology is also a widely used method for producing optically pure isomers. In this procedure, the membrane itself maybe intrinsically enantioselective. This means that the membrane represents a chiral system that separates the desired isomers on the basis of spatial conformation. On the other hand, a membrane separation process may be combined with kinetic resolution by making use of an enantiospecific biocatalyst. That is, the membrane helps in the separation of the product from the substrate on the basis of their chemical properties like solubility.

Enantiospecific catalytic membrane reactors may also be used. These comprise of membrane processes which are advantageous as they have the ability to work in a continuous mode and enormous amounts of material could be processes at once. The competitive production of chiral substances requires a large scale, cheap process for the production and separation of the enantiomers. Eg. Pyridoxal – phosphate dependant lyase and transferase were used as catalyst in the synthesis of L- amino acid via the carbon – carbon bond formation. (Sheldon, 1993)

The widely used enantiospecific membrane reactors are ultrafiltration hollow fibre membrane reactor (Responsible for production of L – phenylalanine by using dehydrogenase catalyst (Schimdt et al, 1987)) immobilized enzyme membrane reactor, packed bed continuous bioreactor, biphasic membrane reactor etc.

Ultra filtration, electrodialysis and membrane extraction are common separation processes that are combined with biotransformation. Matson and Quinn(1979) showed the optimization in production of amino acids enantiomers and studied the separation of L amino acids from the racemate solution by making use of an impregnated liquid membrane alone with an enzyme immobilised membrane. Production of L-phenylalanine from racemic mixture of D, L phenyl lactate was shown by 2 consecutive biotransformation in an enzyme membrane reactor whereby the enzyme and cofactor(NAD/H) had been compartmentalised behind an ultra filtration membrane.(Schmidt et al, 1987).

Intrinsically enantioselective membranes are also widely used. Substances which are optically active can be separated on the basis of there physical stereo selectivity. Polymeric membranes having the enantioselective properties intrinsically may be prepared making use of chiral polymers or by chiral modifications of the achiral porous membrane in the presence of chiral recognition agent like cyclodextrins, cyclophane and oligopeptides. In order to prepare the enantioselective membranes, optically active polyacryl amides and cellulose derivatives may be used. Yoshikawa et al, 1996, showed separation of tryptophan, phenylalanine and alanine by ultra

filtration using the chiral selector which was molecularly imprinted polymeric membranes(DIDE derivatives).

Enzymes have the ability to catalyse a broad spectrum of chemical reactions with great efficiency and selectivity under mild and environmentally friendly conditions. By exploiting the selectivity of enzymes for one form of the enantiomer of a racemic mixture, the enantiomerically enriched compound can be obtained by biocatalytic resolution.(Thomas et al, 2002) Most commonly, the hydrolytic enzyme are used since they display a range of advantages like stability, specificity, no requirement of cofactors. Among hydrolases, lipase is most commonly used because of high enantioselectivity, commercial availability and good stability in various media.(Seung Hwan et al, 2004)

Recently a new technique was introduced to display the peptides and proteins on the surface of gram negative and gram positive bacteria, yeast or mammalian cells. This was done by fusing the peptides to surface anchoring motif; and the technique is known as cell surface display. The cell surface display lipase proved to be an excellent biocatalytic system for the kinetic chiral resolution of the racemic compound.

Recent advances have shown the use of enzymes in the synthesis of optically pure drugs and biologically active compounds. Enzymes have the ability to distinguish between the enantiomers of racemic substrates. Various strategies have been developed to improve the stereoselectivity of resolutions catalysed by the enzyme. This includes modification of the substrate, recycling of the product and altering the reaction conditions. By

making use of these strategies, enzymes with modest stereoselectivity can also be used but only one enantiomer is produced with high yield. Enzyme can catalyse transformations with high region selectivity and chemo selectivity under mild reactions. This is important in the modification of chiral drugs. Eg. Penicillin acylase causes the hydrolysis of benzyl penicillin without affecting the beta lactam ring and allows the industrial preparation of 6-aminopenicillanic acid which is a precursor for many semi synthetic penicillins. Enzymes (hydrolases) have successfully been used in the synthesis of chiral pharmaceuticals, however modern methods of protein engineering and industrial microbiology help in the production of enzymes which are more inexpensive, stable with broad substrate specificity and high stereoselectivity.(Alexey L. Margolin, 1993)

Catalytic asymmetric synthesis is the asymmetric synthesis that is catalysed by chiral (transition) metal complex. The reactions that are involved are Redox transformations or carbon – carbon bond forming processes that complement enzymatic hydrolytic process. The three types of chemo catalysts that exist are heterogenous metal catalyst, homogenous complex and soluble chiral acid or bases. Emil Fisher’s work on asymmetric induction which was based on cyanohydrin synthesis was the first reaction subjected to asymmetric catalysis.

Enantiomerically pure amino acids, amino alcohols, amines, alcohols and epoxides play an important role as intermediates in the agrochemical and pharmaceutical industry whereby high level of purity and a large quantity is required. The enantiomerically pure active compounds help in improving the

economics of the process, thereby leading to reduced quantities applied and less amount of an environmental impact.

Chemical process for the manufacturing of amino acids: Even though asymmetric syntheses of amino acids are known (Michael Breuer et al, 2004), no economical process has been developed. Bucherer – Bergs sub type which is Strecker synthesis was employed for the industrial manufacturing of the racemic amino acids. The alpha amino nitrile is produced from hydrocyanic acid, ammonia and an aldehyde and may be hydrolysed to the amino acid directly or in the presence of carbon dioxide it gets converted into hydantoin. The hydantoin is then subjected to hydrolysis in a basic media to give the racemic amino acid. Another route to the racemic amino acid is amido carbonylation in the presence of a transition metal. Although, there is no commercially viable chemical process for the synthesis of enantiomerically pure amino acid, the production of racemic amino acid is still of great importance because the racemates may be converted to enantiomerically pure compounds by various biocatalytic methods. The catalysts used in the biotransformation are metabolically inactive cells or isolated enzymes. It is the method of choice for the production of enantiomerically pure D- amino acids and various other non natural amino acids. Lyases may be used as biocatalysts in the production of L- Aspartic acid from fumaric acid (Beller et al, 2000). Amino acid dehydrogenase (deaminating amino acid oxido reductase) allows enantioselective biotransformation on an industrial scale. These enzymes have low substrate specificity due to which non natural compounds may also be transformed. In addition, they also require co substrates which help in

supplying the hydride ions for the reduction of Schiff base. There is also a chemo enzymatic method for amino acid synthesis. In this, L- amino acid gets oxidised by L- amino acid oxidase. Imine (intermediate) gets reduced by Pd-C in ammonium formate buffer. In the resulting racemic mixture, only L – enantiomer is utilised by oxidase where as the D- enantiomer accumulates. Therefore, the enantiomeric form of the amino acid which is produced depends entirely on the specificity of the oxidase. The enantiomerically pure amino acid can also be prepared by the racemate resolution. Eg: L and D amino acid can be prepared with the Hydantoinase-carbamoylase system.

Production of carboxylic acids: Carboxylic acid can be isolated from natural sources(chiral pool). Naturally occurring chiral compounds obtained from the chiral pool are an alternative to the synthesis of enantiomerically pure products. An examples of a chiral carboxylic acid that is isolated from the natural sources is L – (+) tartaric acid (Mitsugi et al, 1978). During the fermentation of grape, the isomeric form of tartaric acid separates out as tartarate (potassium hydrogen tartarate). On reacting with calcium chloride or calcium hydroxide and sulphuric acid, isomeric tartaric acid is released; gypsum and yeast residues occur as the by products. Natural carbohydrate building blocks were used for several decades for the preparation of sugar acids which were enantiomerically pure. Another method is the classical chemical synthesis which involves crystallization with enantiomerically pure amines. The enantiomers of the racemic carboxylic acids are known to separate by fractional crystallization of the diastereomeric salts which are formed with the enantiomerically pure amines. Eg: Thiazolidine carboxylic acid (enantiomerically pure), an intermediate in the synthesis of CP-060- S is

isolated by the resolution of racemate with N- benzyl-1-phenylethylamine. (Pompejus et al, 2001)

Production of amines: The chemical process involved is the crystallization with chiral carboxylic acids. Isolation of enantiomerically pure amines can be carried out by the crystallization of diastereomeric salts of chiral carboxylic acids with chiral amines (Jacques et al, 1980). Thus (R) or (S) – 1-phenylethlyamine may be produced on an industrial scale by the crystallization with either (R)- mandelic acid or (S)- malic acid. Mandelic acid was shown to be an important resolving agent for numerous numbers of amines. Dutch resolution is a variant of the classical racemate resolution. In order to reduce the search for an appropriate resolving agent for an amine through combinatorial approach, a mixture of many optically active acids were used. The salt that was precipitated contained several acid anions.

Production of optically active amino alcohols: (S)-2-Aminobutanol is an important amino alcohol intermediate which is used for the synthesis of ethambutol (tuberculostatic)and it must be administered in its enantiomerically pure form as it may lead to blindness. The enantiomerically pure form can be obtained from the racemate by carrying out the crystallization with L-Tartaric acid.(Sheldon et al, 1993)

Production of alcohols: The main process involved was the asymmetric hydrogenation of ketones. Noyori et al showed the development of asymmetric hydrogenation of keto esters and ketones. The catalysts used were ruthenium complexes of binap and derivatives like tol-binap (Akutagawa, 1995)and segphos. The biotechnological process is mainly the

enzyme catalysed resolution. For the resolution of racemate alcohols, enzymatic acylations were developed in early 1980’s. The racemic alcohols are made to react with an acylating agent under enzyme catalysis whereby one enantiomer is unconverted whereas the other enantiomer is esterified. The biocatalysts used are bacterial and fungal lipases.

Production of epoxides: This includes sharpless asymmetric dihydroxylation. The route to the formation of chiral epoxides is based on the optically active diols which may be converted to their respective oxiranes. Another method is the Jacobsen asymmetric epoxidation which is based on (salen) manganese III precatalyst and the hypochlorite is used as the stoichiometric oxidizing agent.

The chemical processes may be compared with the biotransformation with respect to the environmental impact and economic efficiency. The drawbacks of the chemical routes are solvent emission or toxicity of certain compounds. On the other hand, chiral technologies are developing rapidly. Highly versatile technologies and procedures are introduced. Most chiral intermediates are produced in minute quantities. Therefore, the criteria that should be considered for the methods introduced are that they should have a broad substrate spectrum, not require specialised equipment and have a cost effective access to a range of products.

It is not possible to make general conclusions about the superiority of one type of technology in comparison with the others. The most economic technique will depend on their component which is why each case should be investigated individually. However, in the overall process, the chiral step

should be introduced as early as possible but this may be hindered by other factors like racemisation of the unwanted isomer.

Membrane chirotechnology is also an emerging technique having several advantages with respect to the purity of simple isomers, productivity and ease of scale up. These techniques have mainly been used at the laboratory scale. Application on a large scale needs more investment especially in developing the experimental set up rather than investigations which have been carried out on chirality that have been developed in the chromatographic field.