Enantioseparation of D- and L- isomers of Chiral Drugs for Improving their Bioavailability: Some Techniques Including Micellization with Gemini Surfactants

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ABSTRACT
The enantiopure drugs are essential for disease treatment as the human body is amazingly chiral selective. Nearly 50% of drugs are chiral but the pharmacological activity resides with only one isomer, termed as eutomer, whereas the other isomer which is inactive or less potent metabolizes by a different way in the body. This toxic isomer in a racemic drug causes side-effects, genetic disorder or may cause even death if taken in high dosage. Therefore, role of stereochemistry in drug action getting more and more attention of medical practice and one should be good knowledge to make decisions regarding while using single enantiomer of any drug. Most of the drugs used in psychiatric practice are currently available in mixture of enantiomers. For some therapeutics, one particular isomer of chiral drug give better pharmacological results with respect to the target organ, bioavailability in body plasma, low toxicity etc., as compared to racemic mixture of that drug. This article explains the chirality of drugs, the stereochemistry of isomers of chiral drugs, emphasizes the difference in the potential biological and pharmacologic differences between the two enantiomers of a drug, and highlights the novel technique to increase the in-vivo solubility of particularly one isomer of drug, so that the bioavailability of eutomer can be enhanced. This can be done by used of surfactants, which encapsulate the enantiomers of drugs at different extend by micellization.

Key words: Enantioseparation, Micellization, Chiral drugs, in-vivo bioavailability, Pharmacological activity.

INTRODUCTION
The Chirality term was originated and discovered by Louis Pasteur for sodium ammonium tartrate in 1848, and further it was found that most of the biomolecules i.e. carbohydrates, amino acids, nucleosides are chiral in nature. Chirality is the property of chiral compounds, which is due to the presence of chiral carbon in the organic compound i.e. a carbon atom which is attached to four different. Secondly, the compound should be asymmetric in nature so that the mirror image of the compound should not be superimposable with its mirror image. The compounds which are superimposing its mirror image are referred as achiral, and such compounds do not show optical activity. Chirality also found everywhere in our body, including the main building blocks of body i.e. amino acids, proteins and also in carbohydrates, nucleic acid (nucleobases), lipid etc. Chirality is explained as - isomer or dextro-isomer (the isomer which rotate the plane polarized light toward right side) and -isomer or laevo-isomer (which rotate the plane polarized light toward left side). These - and - isomers are termed as enantiomers and they are not super imposable to each other. The equimolar mixture of -
isomer and - isomer is known as racemic mixture. But, now days, d- and l- system of designation of chiral compound is considered as old system, so new absolute configuration designated as R/S – system of nomenclature for optical rotation is used. The chiral carbon of optical active compound is designated as R- (in Latin rectus – right side) or S- (in Latin sinister – left side), in order to describe the stereochemistry of that chiral compound. The R- and S- designation are given by certain rules, based on the priority order of atoms attached to chiral carbon, that priority is given according to their atomic number or atomic masses (in case atomic number is identical). After assigning priority order to all the four atoms or group of atoms, rotate eyes from highest priority group to lowest, in doing so, if eyes rotate towards right, it is considered as R- and if eyes rotate toward left, it is considered as S- isomer, provided the lowest priority group should be away from the observer. If any organic compound or chiral drug contains more than one chiral centre, then the stereochemistry of all the chiral centers should be considered before assigning the final optical activity of that drug or compound. Optical rotation is denoted as (+) for dextrorotatory, (-) for laevorotatory and for racemic mixture. So, enantiomers come in pairs. Both the isomer may have same physical and chemical properties, but differ in rotation of plane polarized light: either to left direction or to right direction. So, these two isomers interact differently in our body. As receptors, enzymes in the body are chiral too, so they behave different way toward chiral drug / compound. Single enantiomers either - or -are also called single isomer or stereoisomer. If that single stereoisomer is active against a particular disease that isomer is called eutomer.

**Importance of Chirality in Drug Molecules**

Only one enantiomer is responsible for all pharmaceutical activities. The therapeutic inactive isomer is considered as a foreign substance which is responsible for undesirable pharmacological effects. But if the active enantiomer exhibits a poor therapeutic value or show toxic behavior, the situation may become even more acute viz. R- and S- enantiomers of thalidomide (Figure 1) (the R and S designation is absolute configurations, by Cahn, Ingold and Prelog, is a way of naming enantiomers by their structures) is a therapeutic-specific pair. The R-enantiomer of this drug is therapeutically active and cause sedation, calming and soothing effect that relieves anxiety and promotes sleep. But, the S-enantiomer of thalidomide may cause teratogen formation, and this was reported to causes serious birth abnormalities in about 2000 children.

There are many other drugs in which one particular enantiomer is active or more potent towards required therapeutic action. Ethambutol: Whereas the (S,S)-(+) -enantiomer is used to treat tuberculosis, the (R,R)-(–) Ethambutol causes blindness. Naproxen: (S)-(+) -naproxen act as analgesic and used to treat arthritis pain, but (R)-(–)-naproxen causes side effects such as liver poisoning. The receptor in the body is also responsible for therapeutic effect such as Penicillin drug is stereo dependent, it act as per the behavior of receptor in the body. This antibiotic must entre by interacting with D-alanine chain of cell walls of bacteria in order to react with and inhibit the transpeptidase enzyme.

Propranolol: the L-isomer of propanolol is a powerful adrenoceptor antagonist, but D-isomer is inactive. However, both isomers are active when they are used as a anesthesia. Methorphan: The L-methorphan which is termed as levomethorphan, is a active opioid analgesic, but, the D-methorphan which is termed as dextromethorphan, is suppress cough actively. Carvedilol: (S)-(–)-isomer of carvedilol is 100 times more potent toward interaction with adrenoceptors as compared to its (R)-(+) -isomer. The S-enantiomer of ofloxacin was found to be reported as 8- to 128-fold more active against both gram-positive and gram-negative bacteria than the R-antipode.¹

**Drugs used as Racemates**

Most of the pharmaceutical compounds are marketed as recemates, mixture of two enantiomers of chiral drugs, each with its own chemistry, receptor affinity and pharmacokinetic profile. Two such drugs are albuterol for asthma and omeprazole for gastoresophageal reflux disease and peptic ulcer were reported.² with improved efficacy, pharmacokinetic and reduced side effects and with almost no drug-drug interaction, by using single isomer instead of racemic mixture. But, sometimes, to get optimal activity, some of them need to be used as recemates i.e. labetalol and nebivolol. Many of the recemates need to be separated into single enantiomer or chirally pure components prefixed as R or S enantiomer.
Classification of drugs: bases on the activity of particular enantiomers

All drugs produce side effects, as the extent of toxicity varies widely between different enantiomers. Many toxic reactions which cause side effects due to their therapeutic effect can be avoided by changing drug dose. Recent interest has focused on the role of the different properties of individual drug enantiomers in causing drug toxicity. The drug which has one chiral carbon, exist in the form of pair of isomers, known as enantiomers, both isomer may be active pharmacologically. However, if the main therapeutic benefit resides with one isomer, several possibilities exist for the other enantiomer - the second enantiomer may be inactive, show qualitatively different effect, an antagonistic effect or greater toxicity.¹

I. Drugs in which one enantiomer is active while the other enantiomer is inactive are: S- Amlodipine is more potent than R- amlodipine towards beta blocking property, Levocetrizine – which is antihistamine, R-enantiomer (levo) is more potent than S-isomer; levofloxacine-anti-bacterial activity resides in the S-enantiomer only. In some cases, the R enantiomer is more potent than S viz. R-Pantoprazole, R-Metoprolol.

II. Drugs in which one isomer is more potent than the other are: Methylphenidate: (R,R)-isomer of methylphenidate-approximately is about ten times more potent than (S,S)-methylphenidate; Ondansetron: R-ondansetron – more potent than the S-enantiomer; Pantaprazole: S-pantoprazole – more potent than the R-enantiomer.

III. Drugs in which beneficial effects reside in one enantiomer, the other enantiomer having antagonistic activity are: Salbutamol: (S) – isomer of this drug is responsible for the bronchodilator activity, but it indirectly involved in antagonizing the benefits of (R)-salbutamol and may have proinflammatory effect; Lipoic acid: the beneficial effects reside with R-lipoic acid, while the corresponding S-form can oppose the action of its R-form.

IV. There are many drugs in which enantiomers have entirely different therapeutic effects. For example; R-isomer of Fluoxetine is used to cure depression while S-isomer is effective for treating migraine; S-Propanolol act as -blocker and membrane stabilizer, whereas R-isomer of propanolol has membrane stabilizing as well as spermicidal properties and can be used to treat hyperthyroidism; R-Sibutramine is effective in treating depression whereas S-Sibutramine is used for curing ejaculatory and erectile dysfunction. Some drugs are there in which only one enantiomer is responsible for beneficial effects whereas the other enantiomer has adverse activity are: S- Amlodipine is a calcium channel blocker (CCB) while R-isomer is inactive as CCB.

Chiral Switching: Switches to active isomer from racemates

Use of single enantiomer instead of racemic mixture of some particular chiral drug, is termed as chiral switching. It involves development of unichiral form of the racemic drug which is already in the market. This is one of the methods to get safe-alternative of drug which exist as racemates. Many of novel single-enantiomer of drugs was developed as such, but there are also important examples of new single-enantiomer drugs derived from ‘chiral switches’ of established racemates.

Methods to get single safer enantiomers of chiral drugs

(a) Single isomer chemical entity (NSCE): the drugs which are developed as a single enantiomers come under this category i.e. enalapril, ramipril, diltiazem, atorvastatin, simvastatin, pravastatin, clopidogrel, L-carnitine, levodopa, d-penicillamine, levetiracetam, and rivastigmine.
(b) Actual chiral switches: Switching from existing racemic drug to one of its isomers to get safer alternative, termed as chiral switches, in other words, these are the drug which were initially marketed as racemic mixtures, but after that one isomer is isolated according to their useful therapeutic effects in our body i.e. escitalopram, esomeprazole, dexibuprofen, dexketoprofen, S-ketamine, levocetrizine, levofloxacine, (R,R)-methylphenidate, levo-leucovorin, levo-bupivacaine, and eszopiclone. Switching from racemates to single isomer covers a wide range of drug categories, such as cetrizine which is antihistamines and ketamine which is anaesthetics. There are some examples of chiral switches done recently to produce more safe and more potent alternative to the existing racemates viz. S-isomers of atenolol, metoprolol, ketamine, amlopidine, zopiclone, omeprazole and pantoprazole, and R-isomer of ondansetron, levosalbutamol, levobupivacaine etc. Chiral switch found to replace many racemate drugs to their safer single-isomer drugs, which are more potent and causes low toxicity. Emcure Pharmaceuticals Limited, Pune is reported to develop a number of single enantiomer (unichiral) drugs, e.g. S-isomers of amlopidine, atenolol, metoprolol, pantoprazole, and R-isomer of ondansetron.

Single enantiomers drugs

The commonly used single-enantiomer drugs are classified as:

Respiratory drugs which are used to prevent, relieve or treat the respiratory diseases viz. Fluticasone propionate...
Methods for separation of enantiomers of chiral drugs: Enantioseparation

Various laboratory techniques are available for enantioseparation. Typically, for very broadly applicable laboratory techniques such as chromatography and capillary electrophoresis high capital investments are required. But still, it is found that for small volumes of single enantiomers needed in early development stages, the high enantioseparation costs by chromatographic methods were still less as compare to the total cost for drug development. Some impressive preparative chiral separations have been demonstrated using centrifugal partition chromatography and simulated moving bed chromatography. Another strategy for chiral separations is based on the use of membrane-based approaches. When applying immobilized selectors in (liquid) membranes, the amount of selector needed can be reduced greatly. Low transport rates through the membranes are the main limitations of this technology.

To achieve a high transport rate, liquid-liquid extraction, diffusion and convection transport mechanisms are used. Liquid-liquid extraction is a well known technology which is easy to operate to fractionate the racemate into its enantiomers. The liquid–liquid extraction can be used for wide range of material, as from small scale such as laboratory separation to bulk scale such as separation in chemical industry, so liquid –liquid extraction is one of the most popular technique used for enantioseparation and is known since 1959. The detail regarding first liquid-liquid enantiosepartion was found in late 1960’s.

Capillary Electrophoresis

Capillary electrophoresis is an analytical technique based on the ionic mobility under the influence of applied electric field. The electrophoretic mobility is dependent upon the charge of the ion, the viscosity, and the atom's radius. The separation of ions depends upon the magnitude of applied electric field, more the field strength, faster the separation of ions. Only ions respond toward electric field, so neutral entities are not affected. The mobility of ion also depends upon the charge carrying by that ion, more the charge more will be the mobility of that particular ion under the influence on applied electric field. Capillary electrophoresis is one the most predominately used technique for the separation of ions as it gives results faster with high accuracy.

The enantioseparation of three chiral drugs with the help of capillary electrophoresis was reported. In this method, an achiral ionic liquid, 1-buty1-3-methylimidazolium chloride ([BMIM] Cl), is used as an additive and β-cyclodextrin (beta-CD) was used as a chiral selector, the enantiomers of chlorpheniramine, the precursor of Chloramphenicol and of Loxacin were separated by capillary zone electrophoresis.

Capillary zone electrophoresis was used for enantiomer separation of set of 59 chiral drugs. Six enantiomeric pair of chiral drugs was separated by using x-cyclodextrin as chiral solvating agent. Initially, the cildinium bromide, oxomemazine and tetryzoline were separated, and then
kетамин, орфенадрин, тропикамид были разделены с дальнейшим оптимизацией процесса.

Метод капиллярной электрофореза для разделения действующих веществ в д- и л-изомерах был предложен. Следующие белки, используемые в этом электрофоре, включают бивальный сывороточный альбумин, сывороточный альбумин и другие виды альбуминов, а также α1-гликопротеид, авидин, рибофлavin-связывающий белок, глюкозид крахмала, пепсин и др., а также другие белки, такие как казеин, сывороточный трансферрин и овоглукокаптивный трансферрин.

**Chromatography: High Performance Liquid Chromatography (HPLC)**

В HPLC ацетатные растворы, используемые в сорбиционном и десорбиционном процессах, были разделены между фазой, в которой ацетатные растворы распределены, и фазой, в которой ацетатные растворы десорбированы. Процесс замедляется по мере прохождения энантиомеров через колонку разделения. Энантиомеры замедляются в различных степенях в зависимости от их связи с фазой. Таким образом, разделение энантиомеров может быть выполнено. За последние 40 лет было создано множество новых хиральных стационарных фаз для газовой и жидкостной хроматографии, которые оказались эффективными для разделения энантиомеров и других различий в хиральной компоненте. Но все еще, разделение энантиомеров соединений, которые имеют более двух хиральных центров, представляет собой вызов. Разделение энантиомеров реобексетина по HPLC было проведено с помощью целлюлозного Трис (3,5-диметилфеноль) карбамат на силикагеле. Разные концентрации смеси н-гексана и пропана-2-ола изучались как мобильная фаза, и оптимальный результат был получен при использовании специфической концентрации н-гексанола и пропана-2-ола в объеме 80%.

**Crystallization**

Кристаллизация является одной из физических методик, используемых для разделения энантиомеров. Кроме того, в резюме рациематов, описанных в разделе 1, может быть использовано для разделения энантиомеров, полученных из асимметрического синтеза, биотрансформации, кинетической резолюции и HPLC. Трех основных типов кристаллизации для энантиомерной резолюции:

- Предпочтительная кристаллизация или разделение энантиомеров по стереоспецифическому росту каждого энантиомера в различных кристаллизаторах. Этот процесс не требует резолюционного агента. Для описания разделения энантиомеров методом предпочтительной кристаллизации в условиях нескольких кристаллизаторов было предложено математическое моделирование.
- Стереосоизомерная кристаллизация - для разрешения пары энантиомеров, используя различного рода реагенты. Эти соли могут быть разрешены в зависимости от их различных свойств. Недавно предложен новый метод, называемый SC (stripping crystallization), который может использоваться для разделения изомеров ибупрофена.
- Катализативная резолюция - в этом методе, реагенты комбинируются с различной скоростью с различными энантиомерами. Недавно предложен новый метод, использующий ко-кристаллизацию в растворе (CCS) для разрешения рациематов.

**Micellization by Surfactants: Enhancing in vivo bioavailability**

Как поверхностно-активные вещества, они обладают двумя свойствами: головная фаза (гидрофобная) хорошо растворима в воде, в то время как хвостовая фаза (гидрофобная) плохо растворима в воде, но хорошо растворима в неполярных растворителях. При добавлении поверхностно-активных веществ в воду, при определенной концентрации они образуют микролиты. Этот процесс называется микролитизацией, и концентрация, при которой образуются микролиты, называется Критической Концентрацией Микролитизации (CMC). Лекарство включается внутрь микролита (рисунок 2) как гидрофобное вещество.

**Figure 2: Drug encapsulating in micelle.**
CONCLUSION

Enantioseparation of chiral drugs by micellization is a novel approach to enhance the bioavailability of drug in the blood stream. Use of single isomer for the treatment of a particular disease is much safer / effective than to use racemic mixture. This reduces the side effect as well as enhances the therapeutic effect at the target cell in the body plasma. The novel synthesized carbohydrate derived surfactants or non-ionic gemini surfactants were explored for recognition of enantiomers of chiral drugs, as these surfactants encapsulate D- and L- enantiomers at different extent. The above discussed surfactants were used to encapsulate the poorly water soluble drugs i.e. Norfloxacin, Glibenclamide and Clofibrate, and enhanced the solubility of these drugs many folds. The increased solubility of these drugs in water, potentially improve their bioavailability, which is required to get desirable therapeutic effect of any particular drug in the body.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.
SUMMARY

Recent drug discovery has led to an increasing number of new drugs with low water solubility and hence poor bioavailability, especially via oral administration. The number of such drug candidates has increased enormously and almost 60% of the new drug candidates have shown poor aqueous solubility in the recent years. Enhancement in their aqueous solubility may improve their bioavailability and thus, reduces side effect, and the dose of the drug can be reduced. Gemini surfactants are new category of surfactants which comprising two conventional surfactants connected via spacer. They have very low critical micellisation concentration (cmcs), so they can encapsulate drugs more effectively. Further, these gemini surfactants can be used for recognition of D- and L-enantiomers of chiral drugs.
Singh, et al., Enantioseparation of D- and L-Isomers of Chiral Drugs

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