Recent Trends in Chiral Separations on Immobilized Polysaccharides CSPs

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Abstract: Polysaccharide CSPs are recognized widely in chiral chromatography but the introduction of immobilized phases (Chiralpak IA, Chiralpak IB and Chiralpak IC columns) is a remarkable achievement. The immobilized CSPs can be used with organic, normal and reversed phase modes; even with prohibited solvents too (tetrahydrofuran, chlorofom, dichloromethane, acetone, 1,4-dioxane, ethylacetate, and certain other ethers). Their susceptibilities to work with a wide range of solvents have increased the range of applications including chiral recognition mechanisms. Besides, these are also useful for monitoring the progress of stereo-specific reactions; normally need prohibited solvents. The present review describes the various aspects of commercial available immobilized chiral columns. Attempts have been made to discuss immobilized polysaccharides CSPs, immobilized *vs* coated CSPs, comparison of immobilized CSPs, method development, optimization, chiral recognition mechanism and applications. The chiral recognition capabilities of commercial columns were in the order of Chiralpak IA > Chiralpak IB > Chiralpak IC columns; but complimentary to each other. Of course, these CSPs are not fully developed and need more advancements and applications. Definitely, the future of immobilized CSPs is quite better. Hopefully, in the coming years they will be the choice of the chromatographers for chiral separations in liquid chromatography.

Keywords: Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiral Recognition Mechanism, Chiral Separations, CSPs, Comparison of Immobilized CSPs, Immobilized Polysaccharides.

INTRODUCTION

It is well established that only one of the enantiomers is pharmaceutically active while the other may be toxic or inactive or ballast. Due to these facts, the whole world is worried and scientists are working to develop optically active pure drugs. Therefore, US FDA [1], European Committee for Proprietary Medicinal Products [2], Health Canada [3], Pharmaceutical and Medical Devices Agencies of Japan [4] have banned the marketing of all racemic drugs. Only optically active pure forms of racemic drugs are sold into the market due to which the demand of chiral separation methods is increasing continuously.

Among some methods, liquid chromatographic modalities are the choice for preparation of optically active drugs. Moreover, chromatographic methods are only capable to monitor the concentrations of enantiomers in biological, environmental and bulk drug samples. This is because of their wide range of applications, ease of operation, selectivities, efficiencies, low limits of detection and reproducibilities. For this purpose, various chiral selectors have been used to prepare Chiral Stationary Phases (CSPs), which include polysaccharides, cyclodextrins, macrocyclic glycopeptide antibiotics, proteins, crown ethers, ligand exchangers, Pirkle's types and several others [2, 5]. Among these, polysaccharides CSPs are the best due to their high selectivities, sensitivities and reproducibilities [2, 5]. Zhang *et al.* [6] reported that more than 95% of racemic compounds have been resolved successfully on these CSPs in chromatographic techniques. The use of polysaccharides for chiral separations has been reviewed by a number of workers [7-10]. During last few years, significant advances have been made in this area and immobilization is one of the most recent developments in these CSPs. Ali *et al.* [11, 12] have reviewed the development of immobilized CSPs about four years back and, now, it's a time to review the status of these phases again. The present article describes the recent developments in immobilized polysaccharides CSPs including a comparison of immobilized CSPs *vs* coated ones, mechanisms of enantio-resolution and applications.

IMMOBILIZED POLYSACCHARIDES CSPs

Cellulose and amylose are the mainly used polysaccharides for preparation of these CSPs. It is because of their good abundance and capabilities for chiral resolution. The degree of polymerization of cellulose ranges from 200-14,000 units of glucose. Similarly, more than 1000 glucose units are found in amylose. The chains of these units lie side by side in a linear fashion in case of cellulose and helical in amylose and, hence, later provides more chiral grooves for enantiomeric resolution. Therefore, amylose is better chiral selector in comparison to cellulose [13]. The native amylose and cellulose are not good chiral selectors, and, hence their derivatives were synthesized by different workers [2]. Presently, amylose and cellulose derivatives in use are tris-benzoate, *tris*-(4-methyl benzoate, trisphenylcarbamate, *tris*-(3,5-dimethylphenylcarbamate, *tris*-(R)-1-phenylethylcarbamate, tris-(S)-1-methylphenylcarbamate etc. These have been commercialized by different names

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such as Chiralcel and Chiralpak for cellulose and amylose, respectively, by Daicel Chemical Industries, Tokyo, Japan. Recently, some other industries such as Kromasil, Macherey Nagel, Knauer and Sepaserve have also introduced some chiral columns. All these chiral columns are of normal and reversed phase modes having normal and reversed phases silica gels, respectively. These columns are prepared by simple adsorption of chiral selectors on silica gels [14]. Therefore, they can be used only with some selective solvents and are not of universal use. In spite of good efficiencies of coated CSPs, they could not resolve some racemates due to some limitations. Only low polar solvents such as alkanes (*n*-pentane, *n*-hexane, *n*-heptane etc.) and alcohols (methanol, ethanol, 2-propanol etc.) or their mixtures, or sometimes, acetonitrile can be used for the chiral separations under normal phase mode. On the other hand, some polar solvents such as tetrahydrofuran (THF), chloroform, dichloromethane, acetone, ethylacetate and methyl *tert*-butyl ether are prohibited with these CSPs and cannot be used [15]. These solvents may be useful to resolve some racemates, which could not be separated by using low polar solvents. Moreover, polar solvents are also required for the determination of the chiral recognition mechanisms using NMR and other spectroscopic techniques [2, 16]. Sometimes, the polar solvents are also required as sample diluents too. Besides, some stereospecific reactions are possible only in polar solvents and, hence, the monitoring of the progress of such reactions is not possible by using coated CSPs. These drawbacks of coated CSPs compel scientists to tackle this problem, which was solved by preparing immobi-lized phases [17-20]. Presently, Chiralpak IA [amylose *tris*-(3,5-dimethylphenylcarbamate)], Chiralpak IB [cellulose *tris*-(3,5-dimethylphenylcarbamate)] and Chiralpak IC [cellulose *tris*-(3,5-dichlorophenylcarbamate)] columns (Fig. **1**), having polysaccharides immobilized on silica gel, are available in the market. These CSPs can be used with a wide range of solvents. Basically, immobilized CSPs are prepared by chemical bonding with silica gel, which make them stable in almost all solvent with their universal use. The preparation of immobilized CSPs comprises derivatization of polysaccharides, reactions of derivatized polysaccharides with derivatized silica gels, purification of the immobilized CSPs followed by their packing into columns. The immobilization of CSPs on silica gel is carried out by different approaches [21]. Normally, the immobilizat-ion procedure is carried out by radical polymerization reaction but it is also possible by photolytic and enzymatic activations. Native silica gel is not effective for immobilization and, hence, derivatized silica gel such as 3-aminopropyl-derivatives is, generally, used [22]. The rate of chemical reaction and the percentage of immobilization depend on the experimental conditions and type of polysaccharide derivatives. The experimental protocol of immobilization procedure is shown in Fig. (**2**).

IMMOBILIZED *VS* COATED CSPs

Enantiomeric resolution by liquid chromatography not only depends on chiral selectors but also controlled by a number of other parameters. Some racemic compounds have been resolved on coated and immobilized polysaccharide chiral stationary phases simultaneously, which were found to be complimentary to each other. On the other hand, few racemates could be resolved on coated CSPs only while others are better resolved on the immobilized ones under the identical chromatographic conditions. But the ability to work with a wide range of solvents is an extra advantage of immobilized CSP in comparison to the coated ones. Tetrahydrofuran (THF), chloroform, dichloromethane, acetone, ethylacetate and methyl *tert*-butyl ether are prohibited solvents for coated CSPs and cannot be used but these solvents can be used easily with immobilized CSPs. Sometimes, the determination of kinetics, pharmacodyna-mics and reaction mechanisms involve above cited solvents. Therefore, coated CSPs are not capable to work under such conditions. Contrarily, immobilized CSPs can be used for such purposes and, consequently, immobilized CSPs have

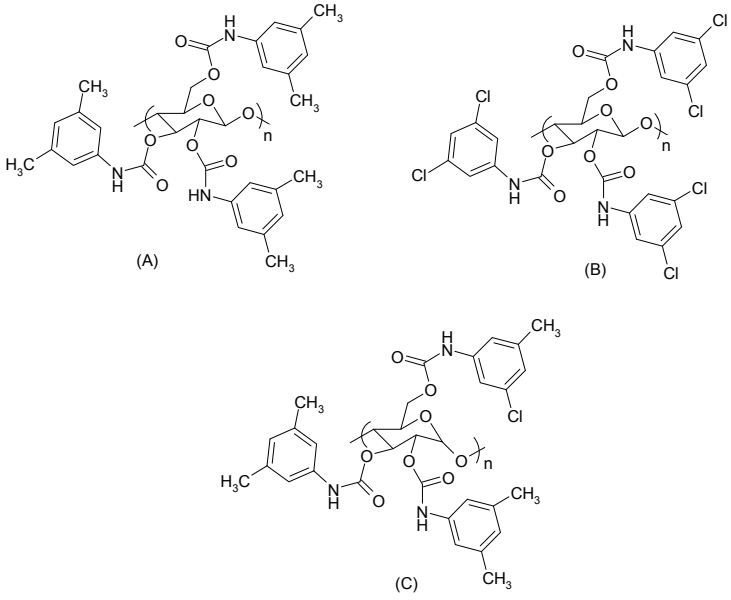

Fig. (1). Chemical structures of (**A**): Chiralpak IA, (**B**): Chiralpak IB and (**C**): Chiralpak IC columns.

more wide range of applications. In spite of added advantages of immobilized CSPs to work with prohibited solvents, sometimes, these have a little bit lower enantio-selectivities in comparison to coated ones. It is due to a slight change/deterioration in high order stereo-specific configurations of the chiral selectors, which occurs during immobilization process. Immobilized CSPs are chemically bonded to silica gel through hydroxyl groups of polysaccharides, which cause an alteration in high order structures and configurations of the polymers; leading to a slight decrease in chiral recognition capabilities [23]. To compare the working capabilities of the coated and immobilized CSPs, some authors attempted to carry out enantio-separation of various racemates on these CSPs.

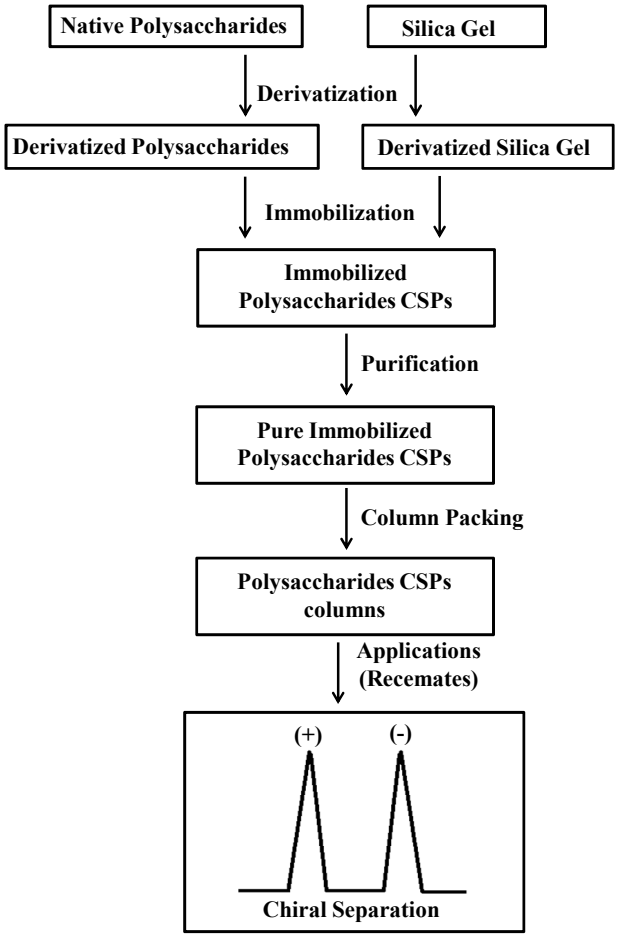

Fig. (2). Protocol for the preparation of immobilized polysaccharide CSPs and their applications.

Okamoto group [22] described slightly poor chiral recognition of immobilized *tris*-(3,5-dimethylphenylcarbamate) cellulose and amylose in comparison to the coated ones. However, the same group [24] reported similar chiral resolution capabilities of immobilized amylose *tris*-(3,5-dimethylphenylcarbamate) and the coated one. Kimata *et al.* [25] immobilized 4-vinylbenzoate cellulose on silica gel and reported slightly lower enantio-selectivity than coated one. Similarly, Yashima *et al.* [23] immobilized CDMPC (cellulose *tris*-(3,5-dimethylphenylcarbamate)) and ADMPC (amylose *tris*-(3,5-dimethylphenylcarbamate)) on silica gel and resolved some racemates by using chloroform, as a component of the mobile phase. Oliveros *et al.* [26, 27] reported immobilization of 10-undecenoate and *tris*-(3,5-dimethylphenylcarbamate) cellulose to several matrices. They carried out the effects of various substituents (of the immobilized CSPs) on chiral resolution of warfarin, lorazepam, oxazepam, tertatolol, propranolol, pindolol, naproxen, flubiprofen and nicardipine racemates. Similarly, other workers also [17,18] immobilized *tris*-(3,5-dimethylphenylcarbamate) and 10-undecenoylcarbamate cellulose, amylose and chitosan on silica gel and reported slightly better enantio-selectivities of cellulose and chitosan derivatives only, while amylose derivatives showed a deterioration in enantio-selectivities.

In 2003, Kubota *et al.* [28] described a quite good chiral resolution of 3,5-dimethylphenylcarbamate cellulose; having a polymerizable vinyl group. The racemates resolved were benzoin, flavanone, 2-phenylcyclohexanone, 2,2,2-trifluoro-1-(9-anthryl)-ethanol and Tröger's base. Chen *et al.* [29] synthesized cellulose phenylcarbamate derivatives and immobilized on silica gel followed by their evaluation of some racemates. The same group [30] also studied efficiency and enantio-selectivity for the developed CSP [immobilized cellulose *tris*-(4-methylbenzoate) (CTMB) derivatives on γ-methacrylatepropylated silica] and reported an increase in efficiency and enantio-selectivity both by increasing the contents of vinyl group on CTMB. Furthermore, they also reported higher efficiency and enantio-selectivity with CSP synthesized by regioselective procedure than non-regioselective one. Zhang *et al.* [6] compared the chiral separation of bupivacaine racemate under identical chromatographic conditions [mobile phase-acetonitrile-diethylamine (100:0.1, v/v)] on Chiralpak AD (coated) and Chiralpak IA (immobilized) columns and the authors reported better resolution on later column. Ghanem *et al.* [31, 32] compared the chiral recognition capabilities of Chiralpak IA column with Chiralpak AD for a variety of racemates and noted a complimentary natures of these two columns i.e. in some cases Chiralpak AD column was found better while in other cases Chiralpak IA gave the best results. Furthermore, Ghanem and Naim [33] compared chiral separations of cyclopropen derivatives on both Chiralpak IA and Chiralpak AD columns using *n*-hexane-2-propanol (90:10 and 99:1, v/v) as mobile phase. It was observed from their work that some derivatives showed good resolution on Chiralpak IA while others on Chiralpak AD column; indicating the complimentary natures of these columns. Thunberg *et al.* [34] reported the separation of forty eight racemates on Chiralpak IA and IB columns and observed slightly poorer enantio-selectivities as compared to their corresponding coated ones.

The above discussion indicates slightly poor chiral capabilities of immobilized CSPs but sometimes, these CSPs gave better results than coated ones, which we observed during our experimental work too. Actually, the above reported papers described the comparisons of enantio-selectivities and efficiencies of these two types of CSPs by using different solvents, which is scientifically incorrect. The comparison should be carried out under the identical chromatographic conditions. But this is not possible as coated CSPs cannot be used with prohibited solvents. Briefly, the comparison of these two types CSPs is not valid.

Sometimes, coated CSPs are better while on other occasions immobilized CSPs work better; depending on the types of racemates and chromatographic conditions. However, we support the use of immobilized CSPs as they can be used without any fear of costly column damage.

COMPARISON OF IMMOBILIZED CSPs

Now, its time to compare the chiral recognition capabilities of three commercialized immobilized columns as some applications are available by using these CSPs. Some papers are available in the literature describing the applications of two or all the three columns for enantiomeric separations of some racemates. We have concluded the chiral recognition capabilities of these columns from the research papers available in the literature. In 2006, Ali *et al.* [15] carried out chiral separations of racemic piperidine-2,6-dione analogues (aminoglutethimide, *p*-nitro-glutethimide, *p*-nitro-5-aminoglutethimide, cyclohexyl aminoglutethimide, phenglutarimide and thalidomide) on Chiralpak IA and Chiralpak IB columns. The resolution factors for Chiralpak IA and Chiralpak IB columns were 1.00-5.33 and 0.33-0.67, respectively; indicating better results on Chiralpak IA column than Chiralpak IB one. Zhang *et al.* [35] used Chiralpak IA and Chiralpak IB, Chiralpak IC columns for resolution of some racemates. General chiral recognition capacities were in the order of Chiralpak IA > Chiralpak IB > Chiralpak IC. This group [36] used all the three columns for efficient method development by using various mobile phases in HPLC and SFC modalities. The authors separated thirty four racemates on these columns. From this paper, no trend was observed but capacity, separation and resolution factors were different for various racemates. Recently, the same group [37] developed an approach for efficient chiral HPLC method by using all the three commercial immobilized columns. Generally, the values of resolution factors in this paper on these columns are in the order of Chiralpak IA > Chiralpak IB > Chiralpak IC. Ciogli, *et al.* [38] used Chiralpak IA and Chiralpak IC columns for enantiomeric resolution of hypericin, pseudohypericin and protohypericin racemate. Protohypericin racemate has shown chiral recognition (separation factors) in the order of Chiralpak IA > Chiralpak IB > Chiralpak IC columns. On the other hand, the trend of enatio-resolution was slightly disturbed from the above order for hypericin and pseudohypericin racemic compounds. Briefly, more or less the chiral recognition capacities of these CSPs are in the order of Chiralpak IA > Chiralpak IB > Chiralpak IC columns; with complimentary natures.

The above discussed order of chiral recognition capacities of these columns (Chiralpak IA, Chiralpak IB and Chiralpak IC) may be explained on the following facts. The chiral selectors in Chiralpak IA, Chiralpak IB and Chiralpak IC are amylose *tris*-(3,5-dimethylphenylcarbamate), cellulose *tris*-(3,5-dimethylphenylcarbamate) and cellulose *tris*-(3,5-dichlorophenylcarbamate) polysaccharides, respectively (Fig. **1**). Chiralpak IA and Chiralpak IB differ only in types of polysaccharides i.e. amylose and cellulose. On the other hand, Chiralpak IB and Chiralpak IC have *tris*-3,5-dimethylpheny and *tris*-3,5-dichlorophenyl carbamates on cellulose units. On the basis of supera-molecular level Chiralpak IA have better chiral recognition capacities than Chiralpak IB due to more helical nature of amylase than cellulose. On the other hand, Chiralpak IB is better than Chiralpak IC column due the presence of chlorine atoms in later chiral selector. It is because of the fact that π-π interactions are the major contributing forces for controlling the chiral resolutions on polysaccharide CSPs [2]. Therefore, the chances of these interactions decrease in Chiralpak IC in comparison to Chiralpak IB column. It is due to the electronegative nature of chlorine atoms, which makes phenyl ring electrons deficient; leading to poor π-π interactions and low chiral recognition capabilities. But it is important to mention here that, sometimes, this order gets changed because chiral resolution is very sensitive process and depends on various chromatographic parameters and the natures of racemates.

OPTIMIZATION OF CHIRAL SEPARATION ON IMMOBILZED CSPs

The chiral resolution is sensitive on polysaccharide CSPs and, hence, the optimization of chromatographic conditions on these phases is a crucial issue. The most important factors, which control enantiomeric resolution include composition, pH and flow rate of the mobile phase, temperature, amount of racemates loaded, structures of solutes etc. We have developed a protocol by which optimization of chiral separation on these CSPs can be achieved easily (Fig. **3**). However, Table **1** also provide clue for the maximum and best resolution of different racemates on immobilized polysaccharide phases. Some workers tried to optimize chiral resolution on these phases, which are discussed below briefly.

Weng *et al.* [39] optimized chiral separation of seven binaphthyl compounds on Chiralpak IA column. The authors optimized chiral resolution by using polar modifiers (normal phase) and column temperatures. As per the authors, 1-propanol was the best modifier. In the same year, Mitchell *et al.* [40] developed the optimization strategies for chiral separation of some racemates on Chiralpak IA, IB and IC columns. Yao *et al.* [41] optimized enantio-separations of 1,1-bi-2-naphthol (BINOL) and its three derivatives on Chiralpak IA. The authors used polar modifiers and column temperatures for optimization purpose. Okamoto and his group [42] optimized chiral resolution of some racemates on 3,5-dimethylphenylcarbamates of cellulose and amylase; bearing 4-(trimethoxysilyl)-phenylcarbamate groups. The authors studied the effect of amount of 4-(trimethoxysilyl)-phenyl groups on immobilization and enantio-separation. These CSPs showed improved chiral resolution by introducing suitable amount of 4-(trimethoxysilyl)-phenyl groups. The authors also studied the solvent durability of the immobilized type chiral packing materials (CPMs) with the eluents containing chloroform and tetrahydrofuran.

Ferretti *et al.* [43] validated enantio-separation of terazosin on Chiralpak IC column under both polar organic and reversed phase modes. The linearity ranged reported was 8.76-26.28 μg/mL for both enantiomers in reversed phase mode. The limits of detection and quantification were 10 and 30 ng/mL, respectively. The intra- and inter-day assay precision was less than 1.66% (RSD). Furthermore, the same group [44] also validated enantiomeric resolution of omeprazole on Chiralpak IA column in both polar organic

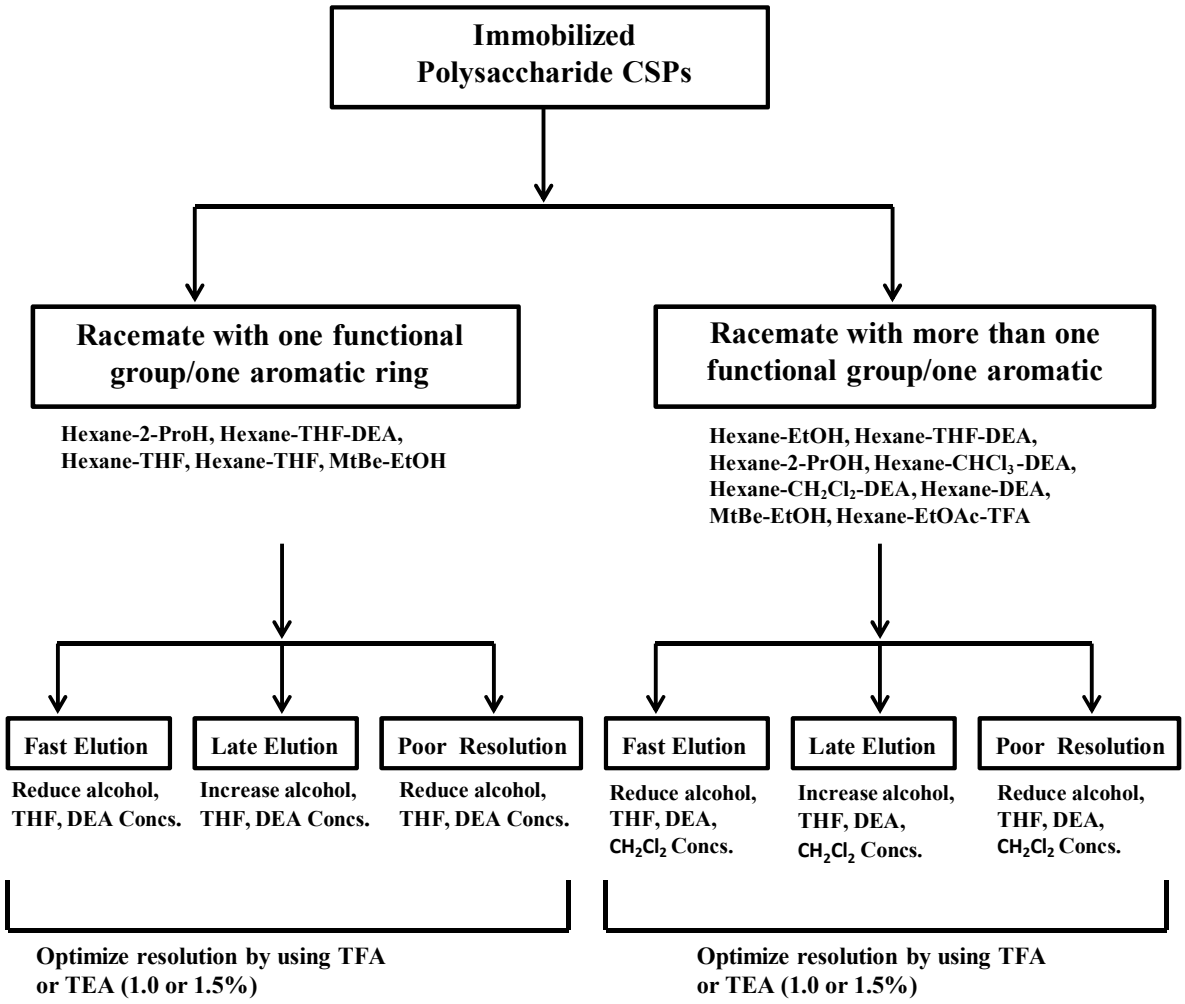

Fig. (3). Protocol for the chiral separations on immobilized polysaccharide CSPs. Note: This is a brief outline of the procedure to follow in developing resolution on immobilized polysaccharide CSPs. However, other optimization by different mobile phases may be used.

Table 1. Suitable Mobile Phases for Immobilized Polysaccharide CSPs

Mobile Phases	Alkane-EtOH	Alkane-2PrOH	Alkane-THF	Alkane-CH_2Cl_2-EtOH	Alkane-MtBE-EtOH
Initial Composition	80/20	80/20	75/25	48/48/4	1/97/2
Optimized Composition	98/2 to 50/50	98/2 to 50/50	95/5 to 0/100	85/15/0 to 0/80/20	80/20 to 0/40/60

CH_2Cl_2: Dichloromethane, EtOH: Ethanol, MtBE: Methyl *tert*-butyl ether, 2-PrOH: 2-Propanol and THF: Tetrahydrofuran.

and normal phase modes. The precision, linearity and accuracy of (R)-enantiomer (as impurity) and recovery of (S)-enantiomer from a pharmaceutical preparation were determined. The authors reported linearity range as 0.5-25 µg/mL for (R)-enantiomer. The limits of detection and quantification were 99 and 333 ng/mL, respectively. The intra and inter-day assay precision was less than 2% (RSD).

CHIRAL RECOGNITION MECHANISM

The chiral recognition mechanism is one of the most important aspects for chiral chromatographers for applying polysaccharides CSPs properly and precisely. But the chiral recognition mechanism at a supera-molecular level is still not clear. The chiral resolution mechanisms on coated and immobilized CSPs are similar. Some experimental data supported that chiral resolution is achieved through different types of bondings on the chiral grooves of polysaccharides CSPs. Most important adsorbing site on the phenylcarbamate derivatives are polar carbamate groups; capable of interacting with racemic compounds by hydrogen bonding with –NH- and >C=O groups and the dipole-dipole interaction on >C=O [45]. Yashima *et al.* [46] concluded that -NH- and >C=O groups are most important bonding sites. Furthermore, these authors extended their work [47] and compared the chiral recognition between cellulose *tris*-(phenylcarbamate) (CTPC) and cellulose *tris*-(3,5-dimethylphenylcarbamate) (CDMPC) using *trans*-stilbene oxide and benzoin as the racemates. Aboul-Enein and Ali [48] carried out studies on the chiral resolution of methylphenidate on polysaccharide CSPs and observed that π-π interactions were important binding forces for the chiral resolution of aromatic racemates.

Table 2. Enantiomeric Resolution of Some Racemates on Immobilized Polysaccharide Based CSPs by HPLC

Racemates	CSPs	Mobile Phases	Refs.
Alprenolol, chlophenadol, chlopheniramine, promethazine, diperodon, 4-fluorophenyl-γ-butyrolactone, laudanosine, propafenone, methaqualone, bupivacaine, EMD 53986, hexobarbital, metalaxyl	Chiralpak IA	Hexane-acetone, hexane-2-propanol, hexane-THF, hexane-toulene-EtOH, MtBE-EtOH, hexane-dichloromethane, ACN-DEA	[6]
Atropisomers of thiazoline derivatives	Chiralpak IA	Hexane-EtOH & hexane-2-propanol	[50]
Ca-sensitizing drug	Chiralpak IA	MeOH/THF	[51]
Cyclopropanes	Chiralpak IA	Hexane-2-PrOH	[52]
3,6-Dihydro-5-[1,2,3,4-tetrahydro-1-(3,4-dimethoxy-benzoyl)-6-quinolyl]-6-methyl-2H-1,3,4-thiadiazin-2-one(EMD 53998) & EMD 53986	Chiralpak IA	Methanol-THF, dichloromethane-THF & methanol-1,4-dioxane	[49]
Lorazepam, glutethimide, bupivacaine, indepamide, suprofen, terfenadine, mephbarbital & flavanone	Chiralpak IA	Hexane-acetone, hexane-1,4-dioxane, hexane-THF-DEA, & other solvents	[53]
Mianserin and a series of aptazepine derivatives	Chiralpak IA	*n*-Hexaneethanol-DEA, *n*-hexane-ethanol-DEA, *n*-hexane-2-propanol-DEA, *n*-hexane-2-propanol-DEA, ethanol-DEA, 2-propanol-DEA, MtBE-2-propanol-DEA, MtBE-2-propanol-DEA, *n*-hexane-dichloromethaneethanol-DEA, *n*-hexane-dichloromethane-2-propanol-DEA, *n*-hexane-EA-2-propanol-DEA & *n*-hexane-EA-2-propanol-DEA	[54]
Monoamine oxidase B inhibitors	Chiralpak IA	MeOH, CH_2Cl_2	[55]
Laudanosine, terfenadine, mephbarbital, oxprenolol, propranolol & hydroxyzine	Chiralpak IB	Hexane-THF-DEA, hexane-$CHCl_3$-EtNA& other solvents	[56]
Aromatic basic racemates	Chiralpak IA & IB	$CHCl_3$-THF	[57]
Piperidine-2,6-dione analogues (aminoglutethimide, *p*-nitro-glutethimide, *p*-nitro-5-aminoglutethimide, cyclohexylaminoglutethimide, phenglutarimide & thalidomide	Chiralpak IA & IB	MtBE-THF, dichloromethane & acetonitrile	[15]
Terazosin	Chiralpak IC	MeOH-DEA	[58]
2,3-O-Benzylidene-dl-threitol, Bucetin, 1-Benzocyclobutene-carbonitrile, 1,5-Dihydroxy-1,2,3,4-tetrahydronaphtalene, 1,5-Dimethyl-4-phenyl-2-imidazolidinone, 5,5-Diphenyl-4-methyl-2-oxazolidinone, Furoin & 1-Indanol	Chiralpak IA, IB & IC	Hexane-2-PrOH	[36]
Derivatised amino acids, acidic & basic compounds	Chiralpak IA, IB & IC	AcN, THF, Buffers	[37]
Hypericin, pseudohypericin & protohypericin	Chiralpak IA, IB & IC	MeOH-CAN-DIPEAA	[59]
5-(Methylphenyl)-oxybutylin phenyldantoin, 1-Benzocyclobutene carbonitrile, 1-Indanol, Orphenadrine, 2-Phenylbutyric acid, Bendroflumethiazide, Metanepherine, Nicardipine & Terbutaline	Chiralpak IA, IB & IC	EtOAc–hexane, MtBE–hexane Hexane–THF–DEA Hexane–2-PrOH–DEA Hexane–THF–DEA Hexane–2-PrOH–DEA Hexane–MtBE–EtOH–AE	[35]

ACN: Acetonitrile, $CHCl_3$: Chloroform, CH_2Cl_2: Dichloromethane, DEA: Diethylamine, EA: Ethyl acetate, EtOAc: Ethyl acetate, EtOH: Ethanol, EtNA: Ethylnitroaniline, MtBE: Methyl *tert*-butyl ether and 2-PrOH: 2-Propanol.

Amylose and cellulose have chiral grooves, which provide chiral pocket to the enantiomers. π-π Interactions occur between phenyl ring of aromatic racemates and the CSP. Besides, the electronegative atoms such as oxygen, nitrogen and halogens of racemates form hydrogen bondings and dipole-dipole induced interactions within these grooves. In addition to these bondings, steric effect also governs the chiral resolution on polysaccharide CSPs. Besides, some other achiral weak bondings like Van der Waal forces may also contribute in the chiral resolution. During chiral resolution, the enantiomers fit stereogenically in the different fashions into the chiral grooves of CSP, which is stabilized by various types of bondings (as discussed above) of different magnitudes and, hence, the resolution of enantiomers is occurred.

APPLICATIONS

Of course, polysaccharide CSPs have a good potential for chiral resolution as about 95% racemates can be resolved on them successfully. Immobilized CSPs have an extra advantage of their use with a wide range of mobile phases including polar organic, normal and reversed phase modes along with normal and prohibited solvents. Prohibited solvents include tetrahydrofuran, chloroform, dichloromethane, acetone, 1,4-dioxane, ethylacetate and methyl *tert*-butyl ether. Some papers are available on chiral resolution of different racemates on immobilized CSPs on analytical scale, which are summarized in Table **2**. Besides, two papers have reported chiral resolution of some racemates at semi-preparative scale. Zhang *et al.* [49] reported preparative chiral separation of a Ca-sensitizing drug (EMD

53986) on Chiralpak IA column. The optimized parameters were sample solubility, enantio-selectivity and preparative productivity. Ferretti *et al.* [43] reported semi-preparative scale (5.3 mg of racemic sample) chiral separation of terazosin on Chiralpak IC column with less than 12 minutes as elution time using methanol-DEA (100:0.1, v/v) as mobile phase. The authors reported ≥99% enantiomeric excess (ee) for both enantiomers.

CONCLUSION

Of course, polysaccharide CSPs have achieved a good reputation in HPLC. But the introduction of immobilized CSPs has increased their application ranges even in moderate drastic conditions. Besides, their use is not restricted to HPLC only, but can be used in other modalities of liquid chromatography. Immobilized CSPs are better than coated ones as they can be used with polar organic, normal and reversed phases even with prohibited solvents too. These may be an important tool to determine chiral recognition mechanisms; which require polar or prohibited solvents, sometimes. For commercial columns, the order of chiral recognition capabilities was observed as Chiralpak IA > Chiralpak IB > Chiralpak IC columns; but complimentary to each other. These CSPs are not fully developed and need for more advancements and applications. Definitely, the future of immobilized CSPs is quite better. Hopefully, in the coming years they will be the choice of the chromatographers for chiral separations in liquid chromatography.

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