

# Immobilized Polysaccharide CSPs: An Advancement in Enantiomeric Separations

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**Abstract:** With the advancement of science and technology chiral chromatography has achieved an important place in analytical science. Coated polysaccharide chiral stationary phases (CSPs) are most popular due to their high chiral recognition power. But during last few years, immobilization of polysaccharide derivatives with silica gel has opened new realms in this area as these CSPs can be used with normal and prohibited solvents (tetrahydrofuran, chloroform, dichloromethane, acetone, 1,4-dioxane, ethylacetate, and certain other ethers). The present article describes status and method protocol of immobilized polysaccharides CSPs for the chiral resolution of different racemates using liquid chromatography. The contents of this article include methods of immobilization, their applications under optimized conditions, enantioselectivities, efficiencies and a comparison of the chiral recognition capabilities of coated vs immobilized CSPs.

## 1. INTRODUCTION

The need and demand of the enantiomeric resolution of drugs, pharmaceuticals and agrochemicals are well established and documented [1-9]. It is due to the different pharmaceutical activities of drugs and toxicities of the pollutants [8-11]. Among many techniques, chromatography has achieved a good status in the chiral separation due to the availability of a wide range of Chiral Stationary Phases (CSPs). Polysaccharide based CSPs are popular due to their capability to resolve about 95% racemates available [6,7,9]. Polysaccharides (cellulose and amylose) are readily available as optically active polymers but native cellulose and amylose are not efficient chiral selectors because of their insufficient optical resolving power. But these are easily converted into a variety of derivatives such as *tris*-esters and *tris*-carbamates. Many polysaccharides based CSPs have been prepared and are commercially available in the form of various columns [12]. Normally, these CSPs are prepared by coating derivatized polysaccharides on the silica support [13] and have good efficiencies for the chiral separations but they are not able to resolve some racemates due to their certain limitations.

Only low or non-polar solvents such as alkane (*n*-pentane, *n*-hexane, *n*-heptane etc.), alcohols (methanol, ethanol, 2-propanol etc.) or, sometimes, acetonitrile can be used as mobile phases due to the coated nature of these CSPs, which result into their limited applications as mobile phase is the major optimizing parameter in chiral chromatography. Therefore, some polar solvents such as tetrahydrofuran (THF), chloroform, dichloromethane, acetone, ethylacetate and methyl *tert*-butyl ether are

prohibited with these CSPs and can not be used [7,13] while these solvents are useful for resolving some racemates, which could not be resolved by using non-polar solvents. Polar solvents are also required for the determination of the chiral recognition mechanisms using NMR and other spectroscopic techniques [7,14]. Besides, sometimes, polar solvents are required as sample diluents. In addition, some stereospecific reactions are possible only in polar solvents, and, hence, the monitoring of the reaction progress is not possible by using coated CSPs. Due to these drawbacks of coated CSPs, the need of chemical bonding between derivatized polysaccharides and silica gels was realized, which is called as immobilization. Some workers attempted to immobilize chiral polysaccharide phases on silica gel [15-23]. Recently, Chiralpak IA [amylose *tris*-(3,5-dimethylphenylcarbamate)] and Chiralpak IB [cellulose *tris*-(3,5-dimethylphenylcarbamate)] columns, having polysaccharides immobilized on silica gel, were launched into the market, which can be used with a wide variety of solvents [24]. In view of the importance of immobilized phases, attempts are made to describe a state-of-art of polysaccharides immobilization and method protocol in this article including immobilization procedure, their applications under optimized conditions, enantioselectivities, efficiencies and a comparison of the chiral recognition capabilities of coated vs immobilized CSPs.

## 2. METHODS DEVELOPMENT

The complete method protocol of chiral resolution on immobilized CSPs comprises the preparation of immobilized CSPs and their applications in the chiral resolution. 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. Chiral recognition power of the developed CSPs is determined by using High Performance liquid chromatography (HPLC) for

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different racemates. The utility of immobilized CSPs may be examined in Capillary-Electrochromatography (CEC), Capillary Electrophoresis (CE) and other modes of liquid chromatography. This protocol is divided into the following sub-headings. The experimental protocol of immobilization procedure is shown in Fig. 1.

### 2.1. Materials and Reagents

Basically, immobilized CSPs are prepared by the reactions of derivatized polysaccharides with silica gels. Therefore, various derivatives of polysaccharides and silica

gels are used for the synthesis of immobilized CSPs. Basically, the types of derivatives of these two components depend on the requirement i.e. type of CSPs. Most commonly used derivatives are cellulose *tris*-(3,5-dimethylphenylcarbamate) (CDMPC), amylose *tris*-(3,5-dimethylphenylcarbamate) (ADMPC), cellulose 3,5-dimethylphenylcarbamate, cellulose 4-vinylphenylcarbamate, cellulose 2-methacryloyloxyethylcarbamate, cellulose 4-vinylbenzoate, cellulose *tris*-(4-methylbenzoate) (CTMB), *tris*-arylcarmate, *tris*-arylesters of polysaccharides and 10-undecenoylcarbamate derivatives of cellulose, amylose and chitosan.

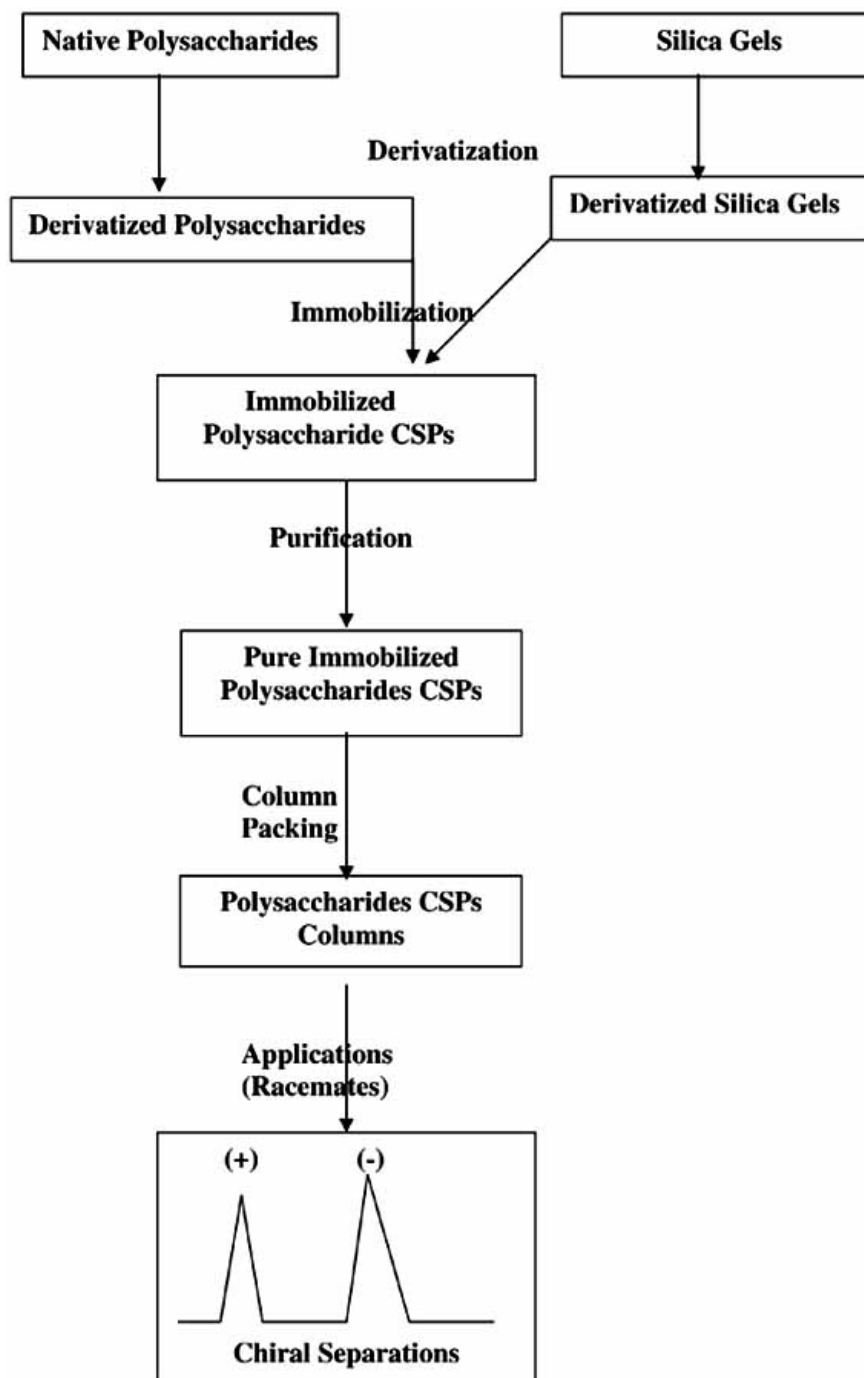


Fig. (1). Protocol for the preparation of immobilized polysaccharide CSPs and their applications.

Similarly, silica gel derivatives used are  $\gamma$ -methacrylate-propylated silica ( $\gamma$ -MAPS), methacryloyldiethyltri-aminopropylated silica (MCDEAPS), 3-aminopropyl silica gel, allyl silica gel, diethylenetriaminopropylated silica (DEAPS), (3-aminopropyl)-triethoxysilane silica gel, 3-aminopropyl-silanized silica gel and  $\gamma$ -aminopropylsilica gel. The other chemicals used are 3,5-dimethylphenyl isocyanate, 4,4'-diphenylmethane diisocyanate, tolylene-2,4-diisocyanate (TDI), 4-vinylbenzoyl chloride, acryloyl chloride, tetrahydrofuran (THF), dichloromethane and other normal solvents.

## 2.2. Equipments

Normally, the derivatization of polysaccharides, silica gels and their immobilization reactions are carried out in reflux assembly, and, hence no special instruments are required for this procedure. However, pH meter, spectrometer (UV, IR and NMR) etc. are used to monitor immobilization reaction. Column packing machine is also required to pack CSPs into columns. HPLC is used to test the chiral recognition capabilities of the developed CSPs. Other accessories such as mobile phase filtration and degasification units must be present into the laboratory.

## 2.3. Immobilization Procedures

Before, immobilization procedure, it is important to decide the bonding sites theoretically between derivatized polysaccharide and silica gel. Many approaches have been used for immobilization purpose [19], which are carried out by radical, photo polymerizations and enzymatic reactions. As in case of polysaccharides, native silica gel is also not effective for immobilization and, hence, derivatized silica gel such as 3-aminopropyl- [15,16] and allyl- [25,26] derivatives are used. The rate of chemical reaction and the percentage of immobilization depend on the experimental conditions and the type of derivatives of polysaccharide and silica gel. For example, 10-undecenylcarboxylate derivatives of polysaccharides show poor reactivity with silica gel in comparison to *tris*-(3,5-dimethylphenylcarbamate) derivatives. The reaction was carried out *via* radical co-polymerization with styrene having a vinyl group at carbon 6<sup>th</sup> position of glucose molecule [21]. We conducted a through search of literature on immobilization and the first attempt on this was carried out by Okamoto *et al.* [15] in 1987 and latter on this technique was improved by further advancements [16,23,25]. A schematic representation of polysaccharides immobilization on silica gel is given in Fig. 2. Basically, Fig.

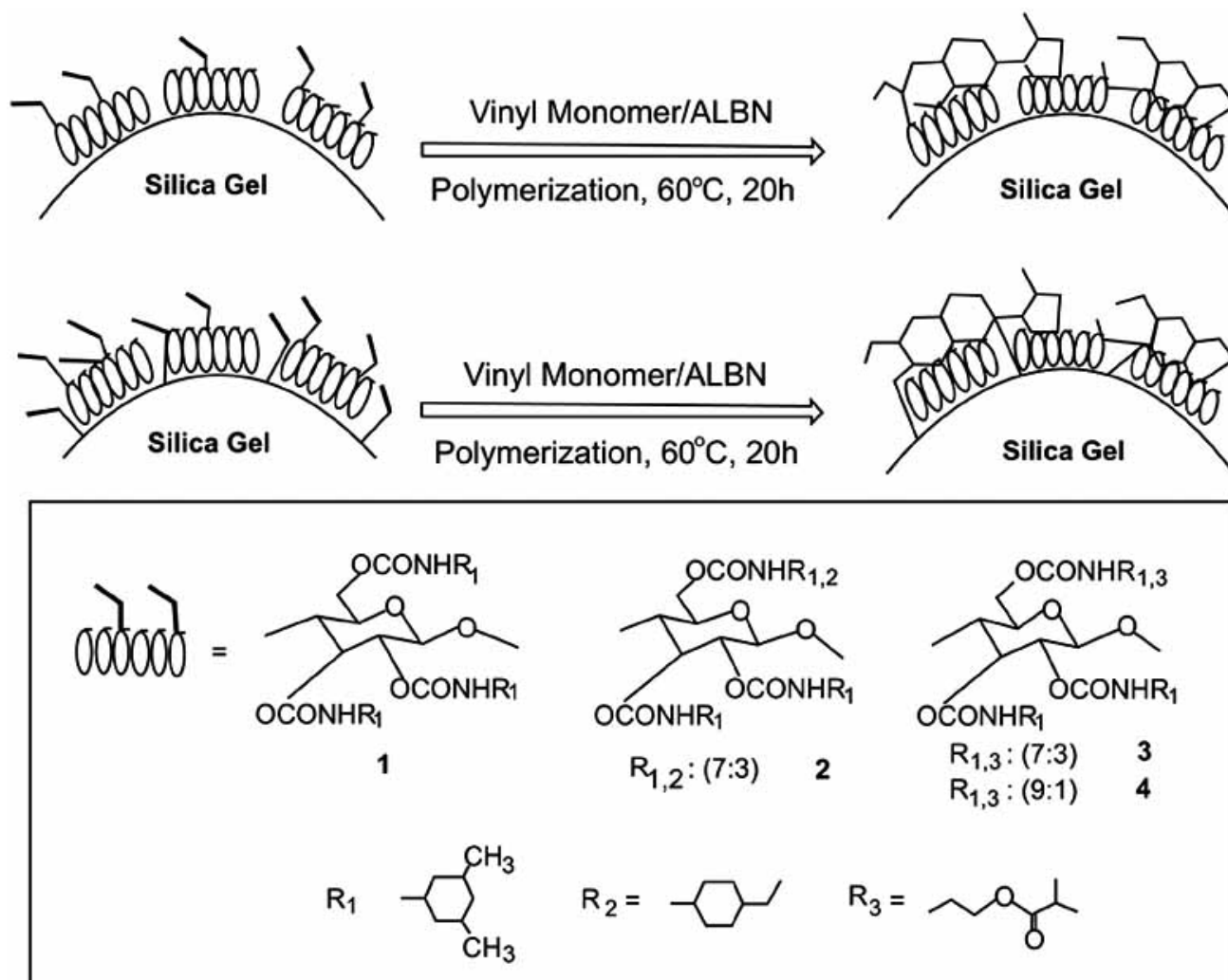


Fig. (2). Schematic representation of immobilization of polysaccharide derivatives on silica gel, AIBN: azobisisobutyronitrile [27].

2 shows an outline of silica gel derivatization followed by immobilization with polysaccharides. First of all silica gel is allowed to react with vinyl monomers at 60 °C for 20 hrs. The second step is the immobilization of this derivatised silica with different derivatives of polysaccharides.

### 2.3.1. Immobilization by Radical Polymerization

Radical polymerization is most common procedure to carry out immobilization. Many workers used this technique to achieve the goal. Kubota *et al.* [21] described the immobilization of cellulose *tris*-(3,5-dimethylphenyl-carbamate), having a vinyl group at carbon 6<sup>th</sup> position of glucose molecule, to silica gel styrene. Recently, same group [27] immobilized cellulose 3,5-dimethylphenylcarbamate derivative, having a polymerizable vinyl group i.e., 4-vinylphenylcarbamate or 2-methacryloyloxyethylcarbamate, with silica gel under various conditions. The concentration and type of vinyl monomer of cellulose derivatives were varied. The authors reported that the introduction of a vinyl group onto silica surface resulted into an efficient immobilization of cellulose phenylcarbamate derivatives. Furthermore, the authors also reported that immobilization became more difficult with low content of vinyl group on the cellulose derivative.

Kimata *et al.* [28] fixed 4-vinylbenzoate of cellulose on  $\gamma$ -aminopropyl silica gel, treated with acryloyl chloride, without any spacer between the matrix and polysaccharide derivatives. Cellulose was fully derivatised with 4-vinylbenzoyl chloride. After coating the cellulose derivative onto the modified silica, a suspension of the resulting material in heptane was heated in the presence of a radical initiator. CSP obtained through this method was stable in THF and dichloromethane. An outline of this procedure is presented in Fig. 3 [19]. Similarly, *tris*-(3,5-dimethylphenyl-carbamate) and 10-undecenoylethylcarbamate derivatives of

cellulose, amylose and chitosan have been immobilized with silica gel using radical polymerization reactions [17-21]. Chen *et al.* [29-32] carried out a remarkable work for the preparation of immobilized polysaccharide CSPs by a radical copolymerization reaction. Positively charged chiral stationary phases (CSPs) were prepared for capillary electrochromatography (CEC) by chemically immobilizing cellulose derivatives onto diethylenetriaminopropylated silica (DEAPS) with tolylene-2,4-diisocyanate (TDI) as a spacer reagent [29]. Similarly, a positively charged chiral stationary phase (CSP) was prepared by chemically immobilizing cellulose 3,5-dimethylphenylcarbamate onto methacryloyldiethylenetriaminopropylated silica (MCD-EAPS) for use in CEC [30]. They [31] have also immobilized cellulose phenylcarbamate derivatives; having methacrylate groups; onto a vinylized silica gel. In another attempt, this group [32] immobilized cellulose *tris*-(4-methylbenzoate) derivatives (CTMB); having methacryloyl groups; on  $\gamma$ -methacrylatepropylated silica ( $\gamma$ -MAPS) through a polymerization reaction. Furthermore, the authors [32] synthesized cellulose *tris*-(4-methylbenzoate) derivatives (CTMB), having methacryloyl groups, *via* regio-selective or nonselective procedures, and were immobilized on  $\gamma$ -methacrylatepropylated silica ( $\gamma$ -MAPS). Oliveros *et al.* [26] reported an immobilization method of four cellulose mixed 10-undecenoate and *tris*-(3,5-dimethylphenylcarbamate) derivatives of cellulose to allyl silica gel. Yashima *et al.* [16] immobilized cellulose *tris*-(3,5-dimethylphenylcarbamate) (CDMPC) and amylose *tris*-(3,5-dimethylphenylcarbamate) (ADMPC) to silica gel by regioselectivity with 4,4'-diphenylmethane diisocyanate as a spacer. ADMPC regioselectively bonded at the 6<sup>th</sup> position to silica gel possesses a high chiral separation capability than that bonded at the 2<sup>nd</sup> or 3<sup>rd</sup> position. For CDMPC, the position of glucose in immobilization on silica gel hardly affected chiral recognition. The enantioselectivities of these CSPs were also

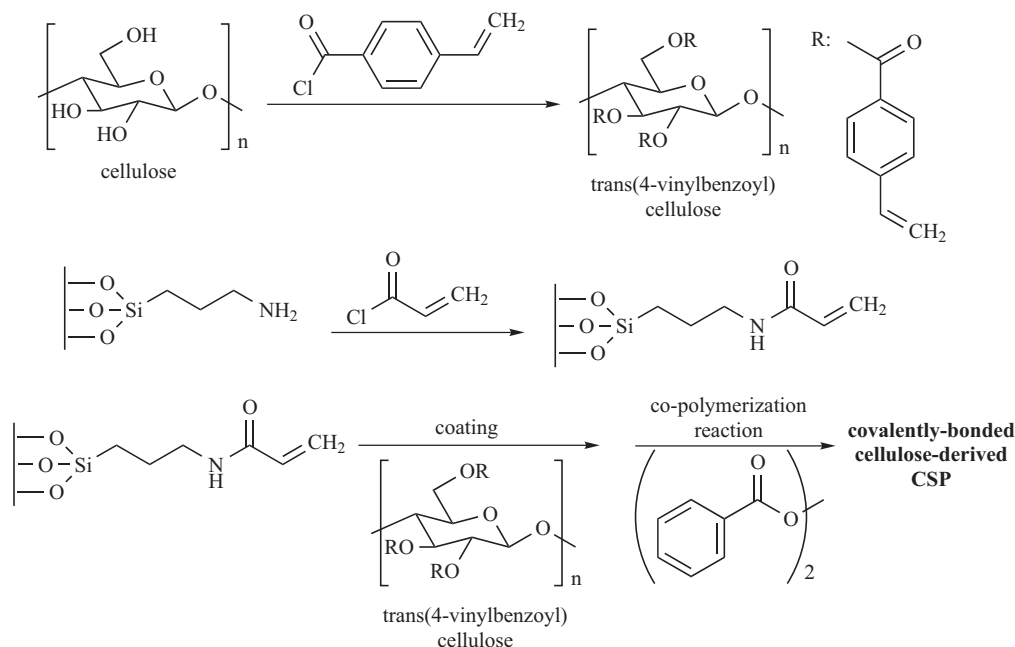


Fig. (3). Immobilization of *tris*-(4-vinylbenzoate) of cellulose onto acrylamidopropyl silica gel [19].

influenced by the amount of the diisocyanate used for immobilization.

### 2.3.2. Immobilization by Enzymatic Reactions

Enzymatic immobilization is the second approach to achieve the stable CSPs but these could not be used wisely due to certain limitations associated with reaction conditions and CSPs obtained. In 1996, Enomoto and Okamoto [23] prepared amylose by enzymatic polymerization of  $\alpha$ -D-glucose-1-phosphate dipotassium, catalyzed by a phosphorylase, using two kinds of the primers derived from maltopentaose. The resultant amylose was converted into its phenylcarbamate derivative and then chemically bonded to silica gel by two enzymatic methods. First method described lactonization of maltopentaose and its reaction with (3-aminopropyl)-triethoxysilane through an amide bond. Amylose, with a desired chain length and molecular weight; was prepared by the enzymatic polymerization following its immobilization with silica gel. Second method described oxidation of maltopentaose at the residual terminal to form potassium gluconate. Amylose end was immobilized to 3-aminopropyl-silanized silica gel through an amide bond. Two amylose conjugated with silica gels, thus, obtained were treated with large excess of 3,5-dimethylphenyl isocyanate to convert their hydroxyl groups to corresponding carbamate residues respectively. CSP derived through method II was found superior than CSP obtained by method I. A schematic representation of the preparation of these CSPs is given in Fig. 4 [19].

### 2.3.3. Immobilization by Photo Polymerization

The third approach of immobilization is through photo or thermal polymerization. Not much work has been carried out by these methods due to the slow reaction rate and poor yields. Francotte and Zhang [20,33,34] reported photo-immobilization of polysaccharide derivatives having no polymerizable group. The authors first prepared coated phases and then converted them into immobilized ones by using photo or thermal reactions between polysaccharides and silica gels. The immobilization occurs by presumably cross linking of the polysaccharide chains. Photo and thermal immobilizations depend on the nature of polysaccharide and silica gel derivatives. As per authors, the exact mechanisms of immobilization are not known. This type of immobilization is shown in Fig. 5 [20].

The regioselective modified polysaccharide CSPs have been reviewed by Felix [35]. The preparations of several regioselective modified polysaccharide derivatives and the selectivities of *tris*-arylcarbamate and *tris*-arylesters of polysaccharides CSPs have been discussed. The use of new substituted derivatives of polysaccharides as chiral stationary phase in HPLC for chiral resolution was also investigated. Franco *et al.* [19] also reviewed the immobilizations of polysaccharide CSPs and discussed their enantioselectivities, efficiencies and stabilities. Recently Ali and Aboul-Enein [36] presented the status of immobilized polysaccharides CSPs in the chiral resolution of different racemates using liquid chromatography. The review comprises discussion on the immobilization methodologies, enantioselectivities, efficiencies and a comparison of chiral recognition

capabilities of coated vs immobilized CSPs. Some applications of immobilized CSPs for the chiral resolution of racemic compounds have also presented.

### 2.4. Preparation of Amylose and Cellulose Immobilized CSPS

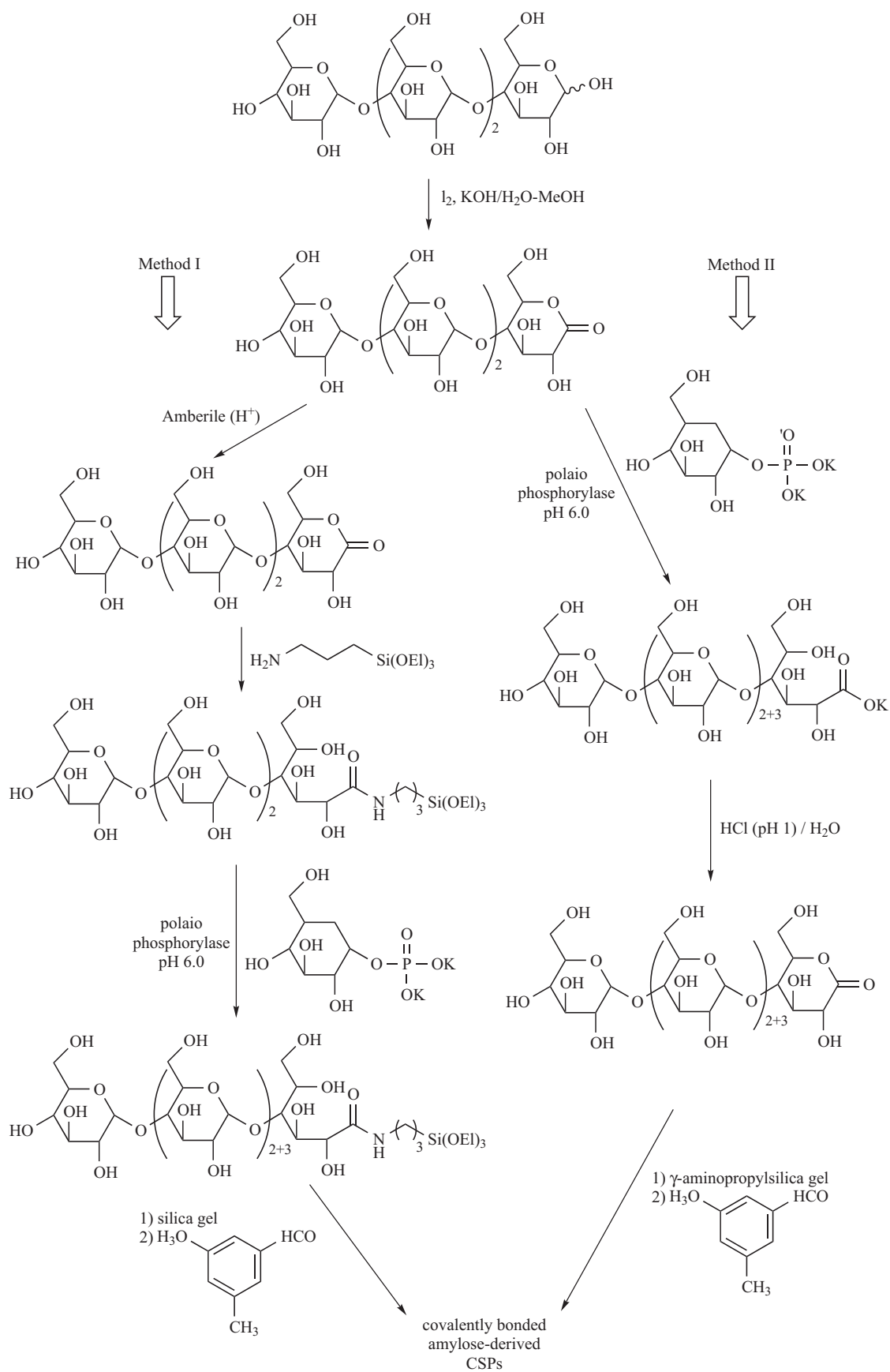
Presently, two immobilized CSPs are effective in chiral separations and have been launched into the market. These CSPs are amylose *tris*-(3,5-dimethylphenylcarbamate) [Chiralpak IA] and cellulose *tris*-(3,5-dimethylphenylcarbamate) [Chiralpak IB] columns. Immobilization is occurred *via* radical polymerization reaction. Therefore, the method protocol of their preparation is summarized below:

1. Treat amylose with 10-undecenoyl chloride (0.4 mol per mol of glucose units) in pyridine and allow the mixture to react at about 100°C for 2 h.
2. Add 3.6 mol of 3,5-dimethylphenyl isocyanate per mol of glucose unit and treat for 24 h at 100°C and finally isolate product as an insoluble fraction in methanol.
3. Redissolve it in chloroform and re-precipitate in methanol.
4. Wash the product with ethanol to remove *N,N*-bis-(3,5-dimethylphenyl)urea, formed as a byproduct, and dry it at room temperature.
5. Purchase allyl silica gel (Nucleosil 100-5; Macherey-Nagel).
6. Dissolve amylose derivative in chloroform or tetrahydrofuran and coat on allyl silica gel (20%, w/w) and evaporate the solvent.
7. Allow to react solid material for 2 h at 100°C in the presence of 2% (w/w) *a,a'*-azobisisobutyronitrile (AIBN).
8. Suspend obtained CSP in chloroform and heat at reflux for 2 h.
9. Filter the suspension, wash with chloroform and acetone and finally dry it.
10. Prepare the slurry of the product obtained in hexane and pack into columns by slurry packing method (Chiralpak IA).

Similarly, cellulose *tris*-(3,5-dimethylphenylcarbamate) CSP can be synthesized and packed into column (Chiralpak IB).

### 2.5. Applications of Immobilized CSPS

Application is the last step of the experimental protocol and, hence, some workers achieved the chiral resolution of different racemates using immobilized polysaccharides CSPs. Non-polar, moderate and high polar solvents were used as mobile phases. Prohibited solvents such as tetrahydrofuran, chloroform, dichloromethane, acetone, 1,4-dioxane, ethylacetate and methyl *tert*-butyl ether have been tested. The chiral separations are very sensitive and, hence, optimization is very important in chiral chromatography. Many parameters are used to control chiral resolution on immobilized CSPs. Most important factors responsible for



**Fig. (4).** Preparation steps of immobilized amylose CSP [19].

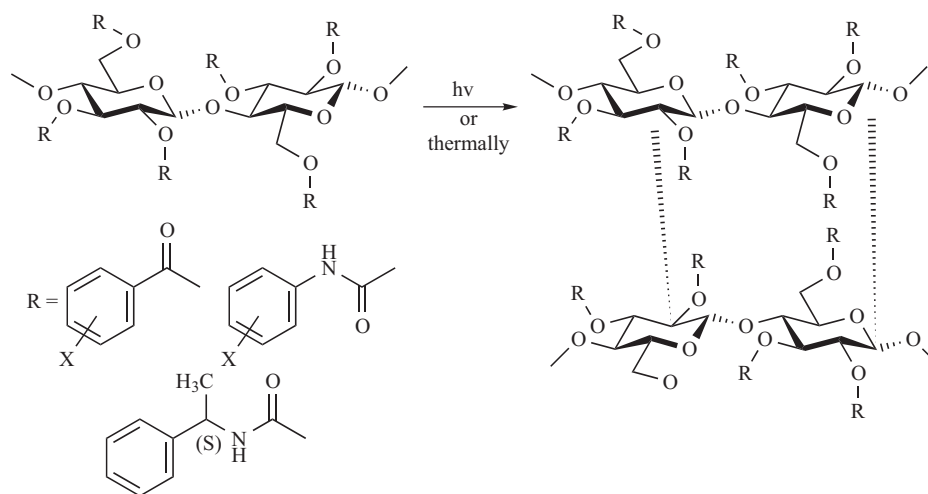
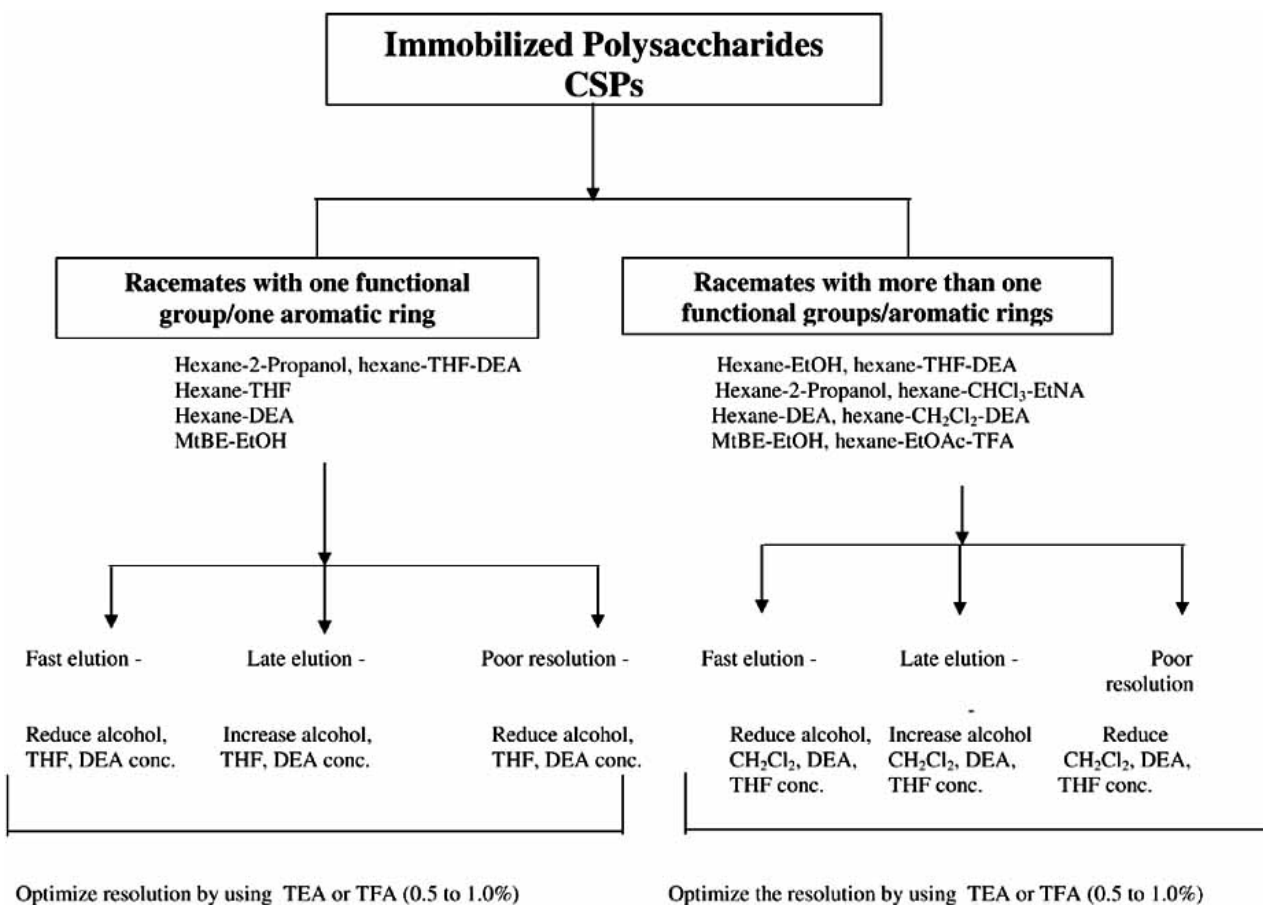


Fig. (5). Process of immobilization of polysaccharide-based CSPs [20].



Note: This is the brief outline of the procedure to follow in developing a resolution on immobilized polysaccharides CSPs. However, other mobile phases may be used.

**Scheme 1.** A protocol for the development and optimization of mobile phases on immobilized polysaccharides based CSPs.

reproducible results are composition, pH and flow rate of mobile phase, temperature and amount loading on the column. To make this article useful for readers, a protocol for the optimization of chiral resolution on immobilized CSPs is given in scheme 1.

Okamoto *et al.* [15] is a pioneer worker in this area and reported enantiomeric resolution of some drugs on immobilized CSPs. Later on same group [16,21,23] described the chiral separations of many racemates. Qin *et al.* [32] tested cellulose *tris*-(4-methylbenzoate)- $\gamma$ -methacrylate-propylated

silica CSP for the chiral resolution of 3-butyl-phthalide, ketoprofen, trans-stilbene oxide, benzoin, benzoic analogue, Troger's base, warfarin, 4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2,2'-dicarboxylate and 4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2-methylcarboxylate-2'-ethylcarboxylate. Duquesne *et al.* [37] used Chiralpak IA column for the separation of thiazoline derivatives atropisomers by using mixtures of hexane and ethanol and hexane and 2-propanol as mobile phases. The authors reported the compatibility of Chiralpak IA column with a wide range of solvents. Cirilli, *et al.* [38] reported chiral separation of mianserin and a series of aptazapine derivatives on coated (Chiralcel OD) and immobilized (Chiralpak IA) CSPs by using normal and polar mobile phases. The authors reported modulation of enantioselectivity degree of Chiralpak IA by using non-conventional mobile phases containing solvent of intermediate polarity, such as dichloromethane and ethyl acetate. The solvent versatility of the Chiralpak IA column permitted mg-scale enantio-separations. Recently, we [39] have resolved several racemic piperidine-2,6-dione analogues [aminoglutethimide, *p*-nitroglutethimide, *p*-nitro-5-aminoglutethimide, cyclohexylaminoglutethimide, phenglutarimide and thalidomide] on Chiralpak IA and Chiralpak IB columns. The non-conventional mobile phases used were methyl-*tert*-butyl ether-THF, 100% dichloromethane and 100% acetonitrile separately.

Chen *et al.* [30] synthesized an immobilized CSP and used for the enantiomeric separation in non-aqueous capillary electrochromatography (CEC). The electroosmotic flow with the prepared CSP could be significantly improved with the introduction of positive charges into the CSP. The separation of enantiomers in CEC has been achieved with mobile phases such as ethanol and hexane-ethanol. Furthermore, the authors investigated the solvent versatility of the immobilized CSP on enantio-separations in CEC and capillary liquid chromatography due to the elimination of dissolution of chiral selector in a number of solvents. Chiral resolution of some enantiomers was improved by using tetrahydrofuran (THF) and chloroform as mobile phase organic modifiers separately. The applications of immobilized polysaccharides CSPs are summarized in Table 1.

### 3. CHIRAL SELECTIVITIES AND EFFICIENCIES

Immobilized CSPs are more stable in a wide range of solvents with complimentary to coated CSPs. Sometimes, immobilized CSPs have better chiral capabilities in comparison to coated CSPs and *vice versa*. Immobilized CSPs are chemically bonded to silica gel through hydroxyl groups of polysaccharides, which will cause an alteration in high order structure and configuration of the polymers resulting into a decrease in their chiral recognition ability [16]. A few reports are available on the selectivities and efficiencies of immobilized CSPs, which are discussed herein briefly. Yashima *et al.* [16] immobilized CDMPC and ADMPC on silica gel and reported good efficiencies of these CSPs using chloroform as mobile phase. Kubota *et al.* [27] reported high chiral recognition capabilities of 3,5-dimethylphenylcarbamate derivative of cellulose using chloroform as mobile phase.

The chiral resolutions of some racemates on 4-vinylbenzoate cellulose based immobilized CSP by using chloroform as one of the components of eluent have been reported by Kimata *et al.* [28]. The resolved racemates were Tröger's base, benzoin, 2-phenylcyclohexanone, 2,2,2-trifluoro-1-(9-anthryl) ethanol and flavanone. The authors reported slightly lower enantioselectivity in comparison to the coated one. Okamoto group [15,16] also reported slightly poor chiral recognition capabilities of cellulose *tris*-(3,5-dimethylphenylcarbamate) derivatives in comparison to the coated ones. However, the same group [23] reported similar chiral resolution capability of immobilized amylose *tris*-(3,5-dimethylphenylcarbamate) with those of the coated one. Kubota *et al.* [27] optimized the chiral recognition efficiency of immobilized cellulose 3,5-dimethylphenylcarbamate derivative by controlling vinyl monomer contents and the type and amount of the vinyl group of the cellulose derivatives. Furthermore, the authors reported a similar chiral recognition capacity of this CSP (having vinyl monomer less than 10%) with coated one; even by using prohibited solvents. Oliveros *et al.* [17,26] studied the effects of various substituents on the immobilized of 10-undecenoate and *tris*-(3,5-dimethylphenylcarbamate) derivatives of cellulose. Their chiral resolution capabilities for several racemates (warfarin, lorazepam, oxazepam, tertatolol, propranolol, pindolol, naproxen, flubiprofen and nicardipine) have been tested. Similarly, other workers [17,21] immobilized *tris*-(3,5-dimethylphenylcarbamate) and 10-undecenoate derivatives of cellulose, amylose and chitosan on silica gel and reported slightly better enantioselectivities of cellulose and chitosan derivatives only, while amylose derivative showed a deterioration in enantioselectivity.

The advantages of broader choice of solvents with covalently bonded cellulose *tris*-(3,5-dimethylphenylcarbamate) (CDMPC) CSP was adopted by Qin *et al.* [40] Structural variation effects of the enantiomers on their retention and separation were also investigated. Furthermore, the same group [31] prepared cellulose phenylcarbamate derivatives; having methacrylate groups immobilized on silica gel; and evaluated their chiral resolution capacities for some racemates. The authors reported a high column efficiency of 30,000-40,000 plates per meter for the eluted peaks. This group [32] also studied efficiency and enantioselectivity for the developed CSP [immobilized cellulose *tris*-(4-methylbenzoate) (CTMB) derivatives on  $\gamma$ -methacrylatepropylated silica] and reported that efficiency and enantioselectivity increased by increasing the contents of vinyl group on CTMB. The authors also advocated high efficiency and enantioselectivity when CSP was prepared by using regioselective procedure in comparison to regiononselective one. Higher efficiency and enantioselectivity of CSP using silica gel II (Fuji, 5  $\mu$ , 30 nm, ca. 150 m<sup>2</sup>/g) was reported by these authors in comparison to silica gel I (Kromasil).

### 4. IMMOBILIZED VS COATED CSPS

As discussed in section 3 immobilized and coated CSPs are complimentary to each other having better resolution possibilities, sometimes, in immobilized or coated depending on the experimental conditions. Of course, the properties and

**Table 1. Chiral Resolution of Different Racemates on Immobilized Polysaccharide Based CSPs Using Liquid Chromatography**

Racemates	CSPs	Mobile Phases	Ref.
Lorazepam, glutethimide, bupivacaine, indapamide, suprofen, terfenadine, mephobarbital & flavanone	Chiralpak IA	Hexane-acetone, hexane-1,4-dioxane, hexane-THF-DEA, & other solvents	[24]
Laudanosine, terfenadine, mephobarbital, oxprenolol, propranolol & hydroxyzine	Chiralpak IB	Hexane-THF-DEA, hexane-CHCl <sub>3</sub> -EtNA & other solvents	[24]
*Warfarin, lorazepam, oxazepam, teratolol, naproxen, flubiprofen, propranolol, pindolol & nicardipine	CSPs 1 to 5	Heptane-2-propanol, heptane-chloroform, pure chloroform & other combinations	[26]
'3-Butyl-phthalide, ketoprofen, <i>trans</i> -stilbene oxide, benzoin, benzoin analogue, Troger's base, warfarin, 4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2,2'-dicarboxylate & 4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2-methylcarboxylate-2'-ethylcarboxylate	Cellulose <i>tris</i> -(4-methylbenzoate)	Moderate & high polar solvents	[32]
3,6-Dihydro-5-[1,2,3,4-tetrahydro-1-(3,4-dimethoxy-benzoyl)-6-quinoly]-6-methyl-2H-1,3,4-thiadiazin-2-one(EMD 53998) & EMD 53986	Chiralpak IA	Methanol-THF, dichloromethane-THF & methanol-1,4-dioxane	[46]
Dimethyl dicarboxy $\alpha$ -biphenyl (DDB) & its analogues	Cellulose <i>tris</i> -(3,5-dimethylphenylcarbamate)	Different kinds of alcohols tetrahydrofuran (THF), chloroform & ternary mobile phases (hexane/2-propanol/THF, hexane/2-propanol/chloroform)	[40]
Alprenolol, chlorhexadol, chlorpheniramine, promethazine, dipiperodon, 4-fluorophenyl- $\gamma$ -butyrolactone, laudanosine, propafenone, methaqualone, bupivacaine, EMD 53986, hexobarbital, metalaxyl, 7,8,9,10-tetrahydrobenzo-( $\alpha$ )pyren-7-ol, ketamine, terfenadine, lorazepam, disopyramide, indapamide, oxazepam, thalidomide, mianserin, dipiperodon, mephobarbital, 1,1'-bi-2-naphthol, 4-benzoyloxy-2-azetidinone, glutethimide, indapamide, temazepam, $\alpha$ -(2,4-DCP)-1H-imidazole-1-ethanol & hydroxyzine	Chiralpak IA	Hexane-acetone, hexane-2-propanol, hexane-THF, hexane-toluene-EtOH, MtBE-EtOH, hexane-dichloromethane, ACN-DEA, hexane-THF-DEA, hexane-CH <sub>2</sub> Cl <sub>2</sub> -DEA, MtBE-THF, MtBE-ACN, MtBE-1,4-dioxane, pure CH <sub>2</sub> Cl <sub>2</sub> & several other combinations	[41]
Acebutolol, alprenolol, atenolol, bisoprolol, bunolol, carazolol, celiprolol, indenolol, metoprolol, nebivolol, oxprenolol, penbutolol, pindolol, propranolol & timolol	Chiralpak IA	Methanol-DEA, Hexane DEA & hexane ethanolamine	[42]
Carprofen, fenoprofen, flubiprofen, ibuprofen, ketoprofen, naproxen, <i>o</i> -methoxymandelic acid, piketoprofen, 2-phenoxypropionic acid, pirofen, rosmarinic acid & warfarin	Chiralpak IA	Hexane-2-PrOH-TFA, hexane-EtOAc-TFA & hexane-2-PrOH	[43]
Five pairs of chiral pyrazole derivatives	Chiralpak IA	EtOH, MeOH, hexane-EtOH, hexane-acetone, hexane-THF, MtBE-EtOH & hexane-CH <sub>2</sub> Cl <sub>2</sub> -EtOH	[44]
Dihydropyrimidines (DHPMs)	Chiralcel OD-I	Hexane-EtOAc	[45]
Piperidine-2,6-dione analogues (aminoglutethimide, <i>p</i> -nitroglutethimide, <i>p</i> -nitro-5-aminoglutethimide, cyclohexylaminoglutethimide, phenglutarimide & thalidomide)	Chiralpak IA & Chiralpak IB	MtBE-THF, dichloromethane & acetonitrile	[39]
Atropisomers of thiazoline derivatives	Chiralpak IA	hexane-EtOH & hexane-2-propanol	[37]
Mianserin and a series of aptazapine derivatives	Chiralpak IA	<i>n</i> -hexaneethanol-DEA, <i>n</i> -hexane-ethanol-DEA, <i>n</i> -hexane-2-propanol-DEA, <i>n</i> -hexane-2-propanol-DEA, ethanol-DEA, 2-propanol-DEA, MtBE-2-propanol-DEA, MtBE-2-propanol-DEA, <i>n</i> -hexane-dichloromethaneethanol-DEA, <i>n</i> -hexane-dichloromethane-2-propanol-DEA, <i>n</i> -hexane-EA-2-propanol-DEA & <i>n</i> -hexane-EA-2-propanol-DEA	[38]
Cyclopropanes	Chiralpak IA	Hexane-2-PrOH	[47]

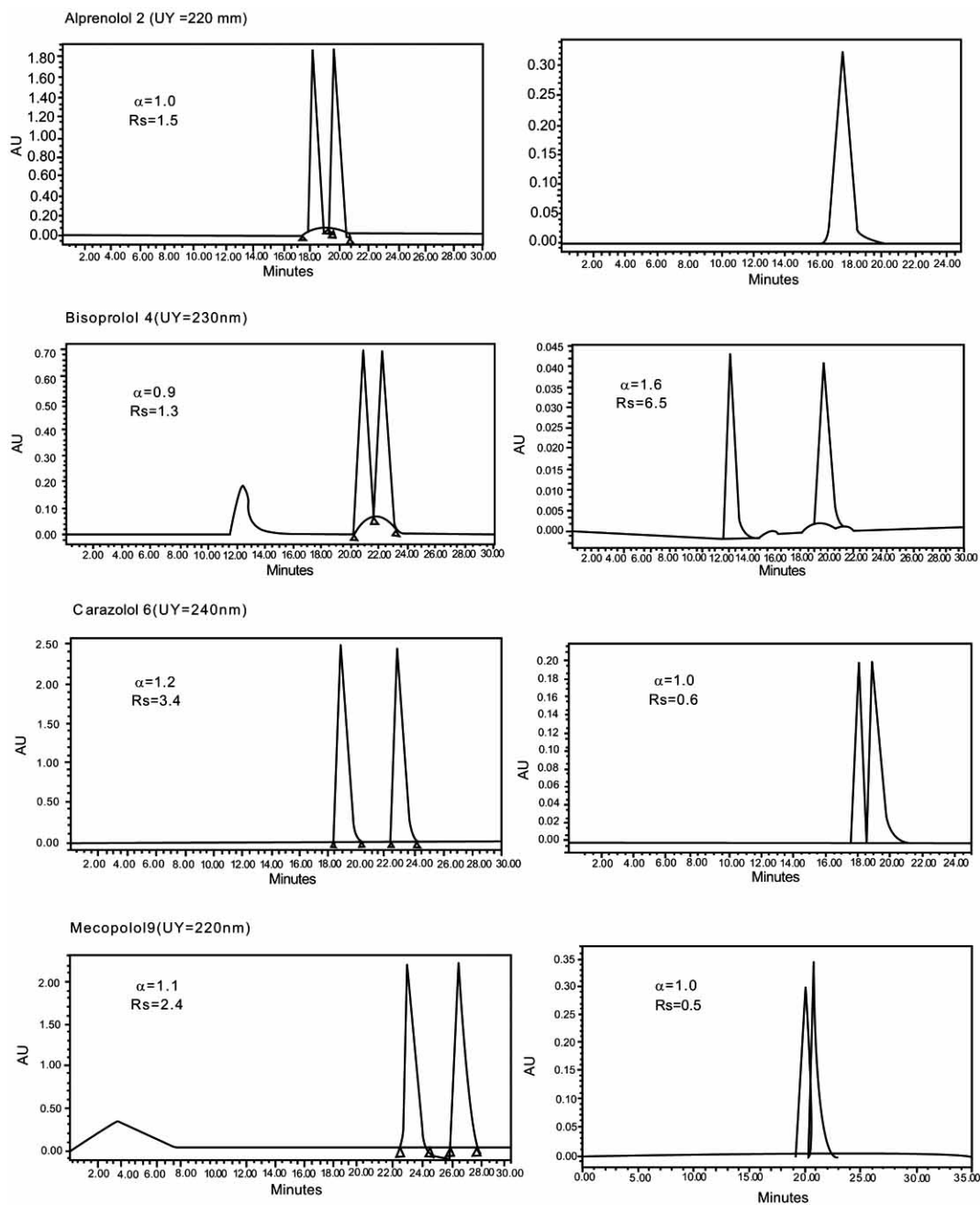
!: Separations by capillary electro-chromatography \*: Immobilized CSPs containing cellulose derivatives of 1:- *tris*-(3,5-dimethylphenylcarbamate), 2:- 4-methylphenylcarbamate, 3:- phenylcarbamate, 4:- 4-chlorophenylcarbamate & 5:- *tris*-(3,5-dichlorophenylcarbamate).

ACN: Acetonitrile, CHCl<sub>3</sub>: Chloroform, CH<sub>2</sub>Cl<sub>2</sub>: Dichloromethane, DEA: Diethylamine, EA: Ethyl acetate, EtOAc: Ethyl acetate, EtOH: Ethanol, EtNA: Ethylnitroaniline, MtBE: Methyl tert-butyl ether and 2-PrOH: 2-Propanol.

the structure of CSPs greatly affect chiral recognition mechanisms. Basically, the chiral recognition by liquid chromatography does not only depend on CSP but also are controlled by many experimental variables. Several racemates have been resolved on coated and immobilized polysaccharide CSPs. Some racemates resolved successfully on coated CSPs only while others on the immobilized ones under the identical chromatographic conditions. However, the ability to use a wide range of solvents is an extra advantage of immobilized CSP.

Some workers compared the capabilities of immobilized and coated CSPs. Zhang *et al.* [41] compared the chiral separation of bupivacaine racemate under identical

chromatographic conditions [mobile phase: acetonitrile-diethylamine (100:0.1, v/v)] on Chiralpak AD and Chiralpak IA columns and the authors reported better resolution on later column. Similarly, Chen *et al.* [31] compared the chiral recognition of immobilized and coated CSPs and reported former CSPs as better due to its stability in polar solvents such as tetrahydrofuran (THF) and chloroform. A wide range of mobile phases including THF and chloroform were tested, which resulted into better chiral resolution. The batch-to-batch and run-to-run reproducibility was also discussed by the authors. Recently, Aboul-Enein *et al.* [42,43] compared the chiral recognition capabilities of Chiralpak IA and Chiralpak AD columns for a variety of racemates and noted



**Fig. (6).** A comparison of the chiral separations of some  $\beta$ -blockers on Chiralpak AD (left) and Chiralpak IA (right) columns using n-hexane-ethanolamine (100:0.1, v/v) as the mobile phase [43].

a complimentary working nature of these two columns. In some cases Chiralpak AD column was found better while in other cases Chiralpak IA gave the best results. A set of racemic acidic drugs on the new immobilized and conventional coated amylose phases were resolved. It was observed that the coated phase (Chiralpak AD) possessed a higher resolving power than the immobilized one (Chiralpak IA). A few racemates, which were not or poorly resolved on the immobilized Chiralpak IA, were most efficiently resolved on the coated Chiralpak AD [42]. Fig. 6 [43] indicates a comparison of the chiral separations of some  $\beta$ -blockers on Chiralpak AD and Chiralpak IA columns, which may be used as an observation of the working capabilities of two types of CSPs. The left and right side chromatograms were obtained by using Chiralpak AD and Chiralpak IA columns respectively. The resolved racemates were alprenolol, bisoprolol, carazolol and metoprolol and its clear from this Figure that out of these four only bisoprolol resolved better on Chiralpak IA in comparison to Chiralpak AD column. Cirilli, *et al.* [44] achieved chiral separations of five pairs of chiral pyrazole derivatives on coated cellulose and amylose CSPs (Chiralpak AD, Chiralcel OJ and Chiralcel OJ-RH) and immobilized amylose based Chiralpak IA column. Chiralpak IA exhibited an excellent chiral resolving ability in normal phase mode and it allowed the enantioseparation of analytes investigated with resolution factors ( $R_s$ ) >20. Authors also used Chiralpak IA at semi-preparative scale in combination with normal phase eluents containing non-standards solvents such as acetone. Under normal-phase conditions, the Chiralpak IA provided to create new enantioselectivity profiles by using non-standard solvents such as acetone, dichloromethane, methyl-tertiary-butyl ether and ethyl acetate. Nadalini, *et al.* [45] reported enantiomeric separation of dihydropyrimidines (DHPMs) coated and immobilized CSPs. Authors compared molecules difference in specific positions of their scaffolds hypotheses concerning the role of individual chemical groups on retention and selectivity. These effects have been quantified in terms of standard Gibbs energy variations. As per authors, no chromatographic measurements have been made under non-linear conditions, which is a clear indication of the potential use of immobilized CSP. Recently, Ali, *et al.* [39] compared the chiral recognition capabilities of Chiralpak IA and Chiralpak IB columns for the resolution of several racemic piperidine-2,6-dione analogues. The resolution factors for Chiralpak IA and Chiralpak IB columns were 1.00 to 5.33 and 0.33 to 0.67 respectively. Chiralpak IA column gave better results than Chiralpak IB column for the reported racemates.

## CONCLUSION

Among various modalities of chromatography chiral chromatography has achieved a respectable status in separation science. Recent development of immobilized CSPs has increased its application widely under various experimental conditions. Basically, immobilization is an innovative area in the chiral separation science as these CSPs can be used with polar solvents. These CSPs can be utilized to monitor certain stereospecific reactions, which are only possible to carry out in polar solvents. Besides, immobilized CSPs may be useful to ascertain chiral recognition

mechanisms. The designing of immobilized polysaccharide CSPs that could be used in a reversed phase mode with solvents that are miscible with water and aqueous buffers would be desirable and increase the potential of these CSPs. The methods of immobilization and the applications of immobilized CSPs are not fully developed yet and are still under progressive stage. We hope that certainly these CSPs will be a great boon in chiral chromatography.

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