# Structure Control of Polysaccharide Derivatives for Efficient Enantioseparation by HPLC

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Introduction

Chromatographic separation (resolution) of enantiomers by high-performance liquid chromatography (HPLC) has considerably advanced in the past two decades and is among the most powerful available methods for determining their purity and for obtaining pure enantiomers. In this method, chiral stationary phases (CSPs) are the key materials for the separation, and many optically active polymers as well as chiral small molecules have been developed to be used as CSPs [1]. Among these CSPs, the cellulose- and amylose-based CSPs may be most attractive from the viewpoints of their wide applicability and easy availability. The polysaccharides are most abundant polymers on the earth and are readily modified to esters and carbamates through the reaction with acid chlorides and isocyanates, respectively. The derivatives have been used as CSPs or packing materials after being coated on macroporus silica gel. Here, the CSPs based on phenylcarbamate derivatives (**1,2**) of these two polysaccharides will be mainly discussed [2]. The immobilization of the derivatives on silica gel will also be discussed.

Cellulose Derivatives (**1**)   Amylose Derivatives (**2**)
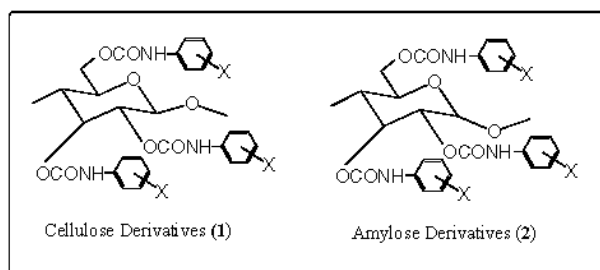
Experimental

The phenylcarbamates of the polysaccharides were prepared by the reaction with an excess of corresponding phenyl isocyanatein pyridine or *N,N*-dimethylacetamide-LiCl and isolated as methnol-insoluble part. The derivatives were dissolved in a solvent and coated on macroporus silica gel (diameter 7 μm, pore size 100 nm) to be used as CSPs. The packing materials were packed in a stainless steel tube (length: 25 cm, inner diameter: 0.46 or 0.20 cm) by slurry method. HPLC analysis was performed using a hexane-2-PrOH (90/10) mixture as eluent.

Results and discussion

Fig. 1 shows the HPLC resolution of trans-stilbene oxide (**3**) on cellulose 3,5-dimethylphenylcarbamate. The (−)- and (+)-enantiomers were eluted at t_1 and t_2, and t_0 is the elution time of a non-retained compound. Then, the separation factor, which stands for the chiral recognition ability of the CSP, is evaluated as $(t_2-t_0)/(t_1-t_0)$. In Fig. 1, this value is 2.27 and $\alpha=1$ means no separation. From the α value, the free energy difference ($\Delta\Delta G^{\neq}$) between the CSP and enantiomers is estimated as $\Delta\Delta G^{\neq}=-RT\ln\alpha$. The α value sufficient for the baseline separation of enantiomers is in many cases 1.2, which corresponds to the $\Delta\Delta G^{\neq}=0.11$ kcal/mol. A tiny energy difference results in the complete resolution.

The chiral recognition ability of the CSPs depends greatly on the substituents on the phenyl group, and the alkyl groups like methyl and t-butyl and halogens often enhance the ability. However, polar groups such as nitro and methoxy reduce the ability [3]. Cellulose trisphenylcarbamate (**1**, X=H) is known to have a regular left-handed 3/2 helical conformation, in which the outside of the chain is surrounded by phenyl groups and the inside of the chain is occupied by polar carbamate and glucose groups as shown in **Fig. 2** [4]. The structural analysis of amylose tris(3,5-dimethylphenylcarbamate) has been done on the basis of NMR data and computer simulation,

which suggest the left-handed 4/3 helical structure [5]. To attain the efficient separation, enantiomers are often requested to interact with the carbamate groups through the polar-polar interaction.

**Fig. 2.** Structure of **1**
(X = H)
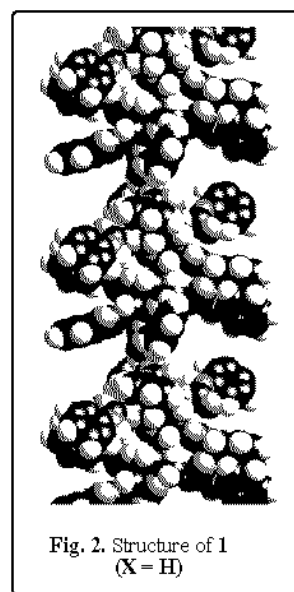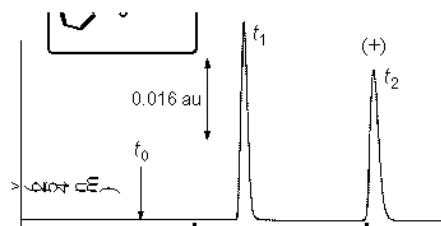
Among each over 50 phenylcarbamates of cellulose and amylose, both 3,5-dimethylphenylcarbamates (commercial name: Chiralcel OD and Chiralpak AD, respectively) exhibit high chiral recognition to a broad range of racemates. Using these two phases, 80-90% of racemates can be resolved.

The 3,5-dimethylphenylcarbamates of oligomers of cellulose, cellobiose (dimer) and cellotetraose (tetramer), showed low chiral recognition different from that of Chiralcel OD, indicating that the high chiral recognition of Chiralcel OD is not due to the single glucose unit, but due to the regular helical polymeric structure [6].

Cellulose Derivatives   Amylose Derivatives

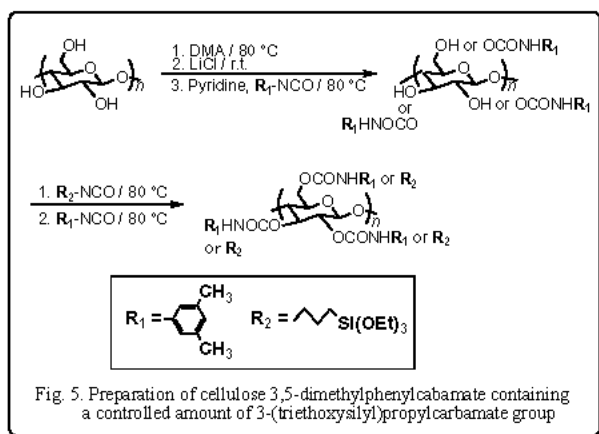**Fig. 3.** Benzylcarbamate derivatives of cellulose and amylose
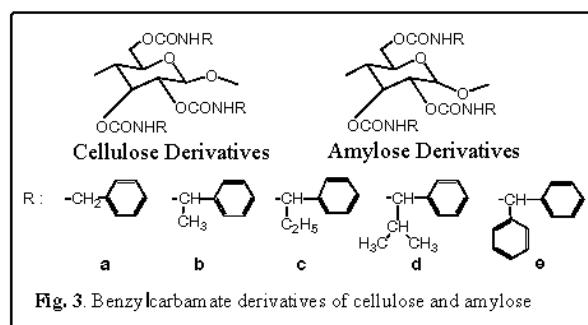
Alkylcarbamates like methyl and isopropyl of the polysaccharides show low chiral recognition. However, the chiral recognition of benzylcarbamate derivatives of cellulose and amylose is significantly influenced by substituents on alpha-position (**Fig. 3**). The derivatives **a**, **d**, and **e** of the both polysaccharides exhibited very low recognition, but the derivatives **b** and **c** resolved many racemates. Only these two derivatives appear to form regular helical structure [7]. The chirality of the substituents also affects the chiral recognition and (*S*) isomer of **b** is superior to the (*R*) isomer in case of the amylose derivative.

Fig. 4. Preparation of immobilized polysaccharide-based CSPs through radical polymerization
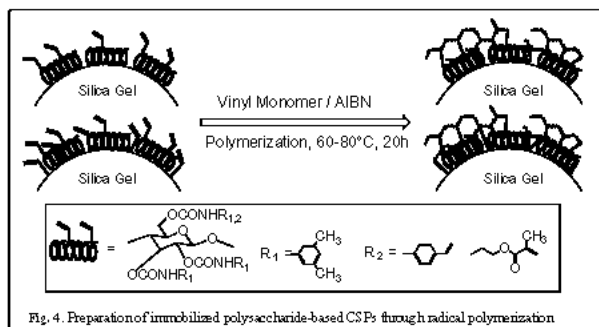
These polysaccharide derivatives have been used as CSPs by being coated on silica gel. Therefore, the solvents that dissolve or swell the derivatives cannot be used as the eluents in HPLC. To overcome this defect, several procedures have been reported for the immobilization of the polysaccharide derivatives [8]. We prepared the Chiralcel OD derivatives bearing partly polymerizable groups like 4-vinylphenylcarbamate and 2-(methacryloyloxy)ethylcarbamate, and immobilized them onto silica gel through radical copolymerization with a vinyl monomer (**Fig. 4**) [9]. The chiral recognition of the obtained materials depends on the amounts and position of the polmerizable groups and a vinyl monomer. The chiral recognition decreased as an increase in the amounts of both the polymerizable groups and the vinyl monomer, although the immobilization efficiency increased with an increase of these amounts. The derivative bearing about 10% polymerizable groups in the total carbamate residues was almost completely immobilized in the presence of 10wt% of styrene monomer. The obtained material can be used with the solvents that dissolve the polysaccharide derivatives.

Recently, we found that the polysaccharide derivatives bearing 5-10% alkoxysilane groups introduced by using 3-(triethoxysilyl)propylisocyanate are also efficiently immobilized through the intermolecular polycondensation [10]. The synthesis and immobilization of this derivative are very straightforward and seem practically valuable (**Fig. 5**).

Fig. 5. Preparation of cellulose 3,5-dimethylphenylcabamate containing a controlled amount of 3-(triethoxysilyl)propylcarbamate group

The immobilization on silica gel was very simply almost quantitatively attained by the treatment of the derivative coated on silica get at 110 °C for 10 min under acidic conditions. When the derivative was coated on the silica gel treated previously with 3-aminopropyl(triethoxy)silane, the immobilization efficiency was

almost the same as that for the untreated silica gel. This indicates that the immobilization proceeds through the polycondensation between the triethoxysilyl groups introduced on the polysaccharide derivatives and that the reaction between the silica gel surface and the polysaccharide derivative is negligible.

Compared with the immobilization through the radical polymerization of vinyl groups, that through the condensation of trialkoxysilane groups seems to more efficiently proceed, if the contents of these groups are similar.

Conclusions

Tris(phenylcarbamate)s, particularly 3,5-dimethylphenyl-carbamtes of cellulose and amylose, exhibit high chiral recognition when used as chiral stationary phases for HPLC. These derivatives have been widely used for the analytical and preparative resolutions of many enantiomers. The CSPs have been prepared by coating them on silica gel. Due to the solubility of the derivatives, the selection of eluents for HPLC has been limited. This defect can be overcome by immobilizing the derivatives on silica gel.

References
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