Synthesis and Characterization of the Chiral Stationary Phase Based on Chitosan

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ABSTRACT: Chitosan was modified with *N*-nicotinoyl-L-phenylalanine and 3,5-dimethylphenylisocyanate to prepare a chiral stationary phase for the high performance liquid chromatography application, especially for the separation of optical isomers of series of α-amino acids. The modified chitosan with *N*-nicotinoyl-L-phenylalanine group and 3,5-dimethylphenylcarbamate group was characterized with several analytical methods such as differential scanning calorimeter, thermo gravimetric analyzer, X-ray diffractometer, and HPLC; its solubility, thermal property, and chiral separation performance were studied. Contrary to chitosan, the modified chitosan prepared in this study showed good solubility in several organic solvents and was easy to handle to coat the silica particles to prepare chiral HPLC column. It showed good chiral separation capabilities. It was also thermally stable at over 100°C, suggesting potential wide operating temperature range of its chiral HPLC column. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 2989–2996, 2007

Key words: chitosan; chiral separation; chiral stationary phase; HPLC; optical isomer

INTRODUCTION

With increasing importance of optical active compounds in the application field of drug,[1,2] pesticide, and food industries, the preparation and application of those compounds have widely been studied. Since its development by the Pasteur, the separation of racemate by the optical active compounds has been known as one of the most important methods for the separation of optical isomers. However, it is not easy to separate optical isomers by the conventional membrane technology, because they have the same molecular size and the same polarity with each other. Among the methods for the optical separation using optical active compounds, it is atypical to use the chiral stationary phase (CSP) prepared from optical compounds for the HPLC.

Enantioseparation by HPLC has advanced considerably in the past decade and many CSP have been prepared,[3,4] and some of those are commercialized and have been used not only for determining optical purity of enantiomers but also for obtaining optically pure enantiomers.[5,6]

Historically, natural polymers, such as cellulose or starch components, were the first to be used as chromatographic chiral selectors because of their inherent chiral nature and ready availability. The resolving ability of polysaccharides, in paticular cellulose, was first observed in paper chromatography when a racemic amino acid gave two spots.[7,8] This led to further use of cellulose and other polysaccharides, mainly amylose, as the chiral starting material in the preparation of selectors to be used in CSPs.[9] The CSPs consisting of phenylcarbamate derivatives of polysaccharides such as cellulose and amylose appear to be one of the most useful CSPs.[10,11] In particular, 3,5-dimethyl- or 3,5-dichlorophenylcarbamate derivatives of cellulose and amylose show high chiral recognition.[12] Some other polysaccharides, such as xylan, dextran, chitin or chitosan, have occasionally been used as starting material for the preparation of CSPs. However, the trisphenylaminocarbonyl and the 3,5-dimethylphenylaminocarbonyl derivatives of chitosan,[13,14] and the phenylcarbamate and 3,5-dimethylphenylcarbamate of the closely related chitin are the only derivatives described as chromatographic chiral selectors.[15]

In this study, chitosan, which has good enantioselectivity and is convenient for chemical modifications at the free amino groups, was used as a starting material for the preparation of a CSP. Two kinds of such side groups as 3,5-dimethylphenylcarbamate groups and *N*-nicotinoylphenylalanine of chitosan were prepared and their chiral recognition abilities as a CSP were evaluated by HPLC.

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EXPERIMENTAL

Materials

All reagents were obtained from commercial sources. As an example, phthalic anhydride, hydrazine monohydrate, *N*-hydroxysuccinimide, L-phenylalanine, 1-hydroxybenzotriazole-6-sulfonamidomethyl-hydrochloride (HOBt · H_2O) and 1-ethyl-3-(dimethyl-aminopropyl)carbodiimide hydrochloride (EDC · HCl) were purchased from the Aldrich Co. (Milwaukee, WI). Triphenylmethyl chloride, nicotinic acid, and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from the Tokyo Kasei Kogyo Co. (Tokyo, Japan). Low molecular weight chitosan [viscosity (Brookfield, 1% solution in 1% acetic acid): 20–200 cps] with a degree of deacetylation of 75 ~ 85% was purchased from the Aldrich Co. Dimethyl formamide (DMF), pyridine, and dimethyl acetate (DMAc) used as solvents of reaction were purchased from the Aldrich Co., and used without further purification. 1,2-dimethoxyethane, dichloromethane, and dichloroacetic acid were purchased from the Tokyo Kasei Kogyo Co. Microporous spherical silica gel with 5 μm of particle size was kindly supplied by RS Tech (OP-5100-NH_2 Silica, Daejon, Korea) and used for the column packing. All solvents used in the preparation of CSPs were of analytical reagent grade. Solvents used in the chromatographic experiments were all HPLC grade. Racemates shown in Figure 1 were all commercially available ones from Aldrich Co., or were prepared by the usual method.[16]

Instrumentals

Chemical structures of polymers and monomers were characterized, using FTIR spectrophotometer (Bio-Rad, Digilab Division, Model FT-80, FTIR), ^{1}H NMR (BRUKER DRX-300). Elemental analyses were carried out using a CE instruments EA 1110 C, H, N analyzer. Thermal properties of the polymers were studied with a Differential Scanning Calorimeter (DSC) (Dupont, Model 910 DSC) and a thermal gravimetric analysis (TGA) (TA instruments TGA 2950); Samples were analyzed under continuous flow of dry nitrogen gas (50 cc/min) at a heating rate of 5°C/min from 5 to 150°C on DSC. Heating was done at a rate of 10°C/min on TGA. The crystallinity and intermolecular distances of the polymers were studied with an X-ray diffractometer (XRD) (Model D/MAX βB Ri-gaku) using nikel-filtered Cu-kα radiation with a wavelength of 1.54 Å. Chromatographic experiments were performed on an HPLC system consisting of a Dionex Pump Series P 580, Dionex Column Thermostat STH 585, equipped with a Dionex UVD 170U/340U UV/VIS Detector; the volume of sample injected was 3 μL The void volume was determined using tri-*tert*-butylbenzene.

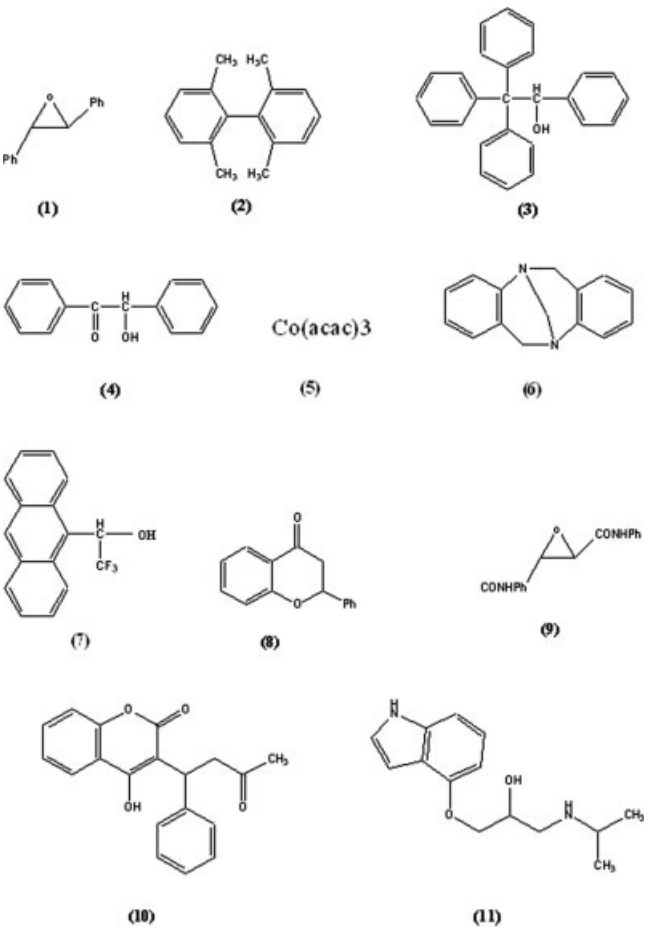
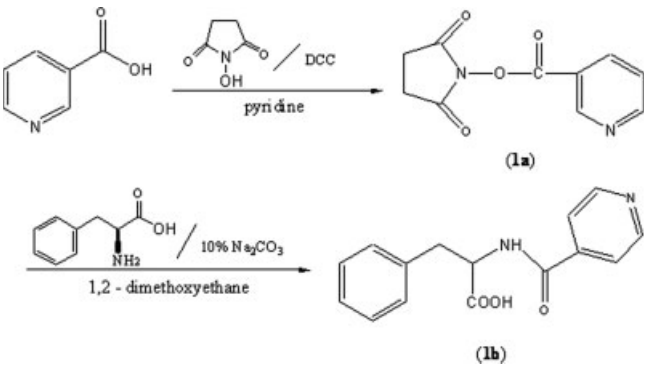


Figure 1 Structures of racemic compounds used for the test.

Synthesis

Nicotinic acid *N*-hydroxysuccinimide ester(1a) (Scheme 1)

21.1 g (0.10 mol) of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 11.5 g (0.10 mol) of *N*-hydroxysuccinimide were added into a solution of 12.3 g (0.10 mol) of nicotinic acid in 200 mL of pyridine, and the mixture was stirred at a room temperature. After 20 h of stirring, the mixture was filtered and filtrate was concentrated under reduced pressure. The solid obtained was then dissolved in 50 mL of chloroform in separation funnel, followed by the addition of 50 mL of distilled water to remove the side product that would be dissolved into water. After shaking the separation funnel for a while, it was kept still until chloroform and water layers were separated, then the water layer was removed. The chloroform solution was then dried over sodium sulfate and evaporated under reduced pressure to give a white solid. The solid was recrystallized from ethanol to give a white needles solid (16.3 g, yield: 74.1%); m.p.: 137 ~ 138°C. IR (KBr): 1798, 1775 and 1725 (C=O), 1590 (arom), 1205 (C—O—C), 723 (pyr) cm^{-1}.



Scheme 1 Reaction scheme for the syntheses of Nicotinic acid *N*-hydroxysuccinimide ester (**1a**) and *N*-nicotinoyl-L-phenylalanine (**1b**).

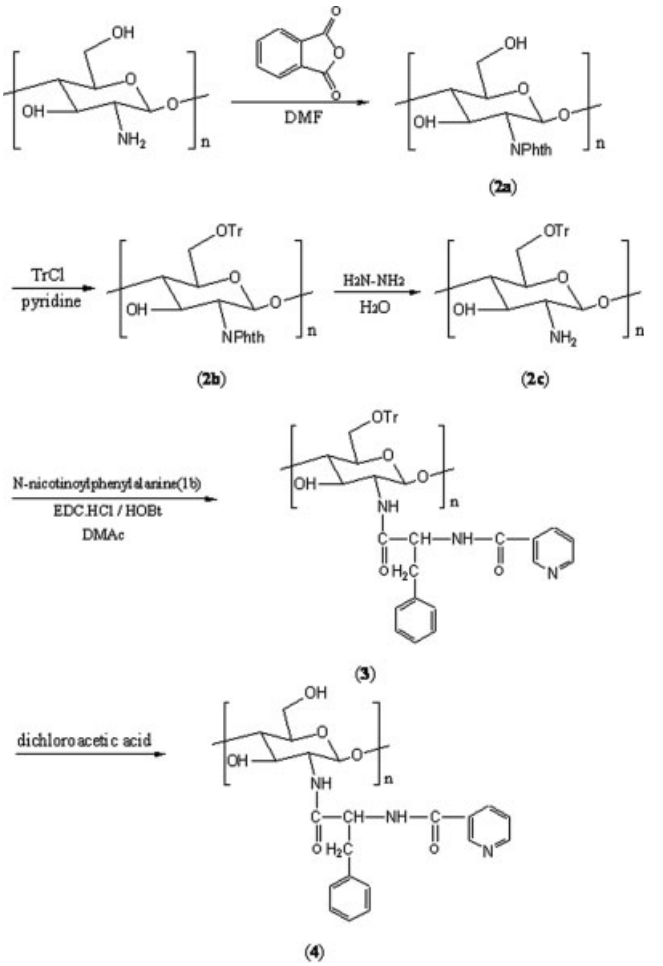
^{1}H NMR (300 MHz, $CDCl_3$): δ 2.94 (s, 4H, CH_2), 7.49 (m, 1H, pry5-H), 8.39 (d, 1H, pry4-H), 8.91 (d, 1H, pry6-H), 9.33 (d, 1H, pry2-H). Anal. Calcd. for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.64; N, 12.73. Found: C, 54.97; H, 3.74; N, 13.05.

N-nicotinoyl-L-phenylalanine(1b) (Scheme 1)

5 g (22.7 mmol) of Nicotinic acid *N*-hydroxysuccinimide ester was dissolved in 250 mL of 1,2-dimethoxyethane, and the solution was cooled in an ice-water bath. A solution of 3.75 g (22.7 mmol) of L-phenylalanine in 100 mL of 10% aqueous sodium carbonate was added drop-wise over a period of 30 min. After stirring at 0°C for 2 h, the mixture was stirred at room temperature for another 14 h. It was then filtered and the filtrate was concentrated to 100 mL under reduced pressure. The solution was acidified to pH 4 using 10% citric acid and extracted with 500 mL of ethyl acetate. The extract was dried with sodium sulfate and evaporated under reduced pressure to give a white solid. The resulting solid was recrystallized from ethanol to give a white small crystals (4.65 g, yield: 75.86%); IR (KBr): 3332 (NH), 1730 (COOH), 1643 (amide), 1600 (arom), 1538 (amide) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.47 (m, 1H, CH_2), 3.72 (m, 1H, CH_2), 5.63 (m, 1H, CH), 7.20–7.58 (m, 6H, ph, pry5-H), 8.33 (d, J-7.9 Hz, 1H, pry4-H), 8.71 (d, J = 5.5 Hz, 1H, pry6-H), 9.52 (s, 1H, pry2-H), 9.70 (d, 1H, NH). Anal. Calcd. for $C_{15}H_{14}N_2O_3$: C, 66.7; H, 5.19; N, 10.37. Found: C, 67.04; H, 5.39; N, 10.58., T_m: 176 ~ 177°C.

6-*o*-tritylchitosan(2c) (Scheme 2)

5 g (31.0 mmol) of Chitosan and 13.8 g (93.1 mmol) of phthalic anhydride in 100 mL of DMF was heated with stirring at 130°C under a nitrogen atmosphere. After 5 ~ 7 h of heating, the mixture became a clear and viscous solution. The precipitants obtained by pouring the solution into ice water was collected by filtration, successively washed completely by soxhlet's extraction with ethanol, and dried under vacuum to give a dark brown product (**2a**) of 5.3 g (yield: 58.37%). To a solution of 5.0 g of phthaloylchitosan in pyridine 75 mL was added a threefold excess triphenylmethyl chloride and the solution was stirred for 24 h at 80°C under a nitrogen atmosphere. The mixture was then poured into ethanol. The precipitant was successively washed with ethanol and ether, and vacuum dried to give a pale gray product (**2b**) of 8.3 g (90.66%). A mixture of (**2b**) 6.1 g (11.43 mmol), hydrazine monohydrate 33 mL, and water 100 mL was heated with stirring for 15 h at 100°C under a nitrogen atmosphere. After cooling, the mixture was then diluted with 100 mL of water and evaporated with a rotary evaporator. This procedure was repeated three times. The residue was resuspended in water, and the precipitatant was filtered. It was washed with ethanol and ethyl ether and vacuum dried to give a pale tan powders (**2c**) 4.9 g (yield: 89.67%); T_g: 110°C. IR (KBr): 3400 (OH),



Scheme 2 Reaction scheme for the syntheses of 6-*o*-tritylchitosan (**2c**) and *N*-(*N*-nicotinoyl-L-phenylalanine)-chitosan (4).

3059 (arom), 2878 (cyclic alkane), 1595 (amine), 1156 (ether) cm^{-1}.

N-(*N*-nicotinoyl-L-phenylalanine)-chitosan(4) (Scheme 2)

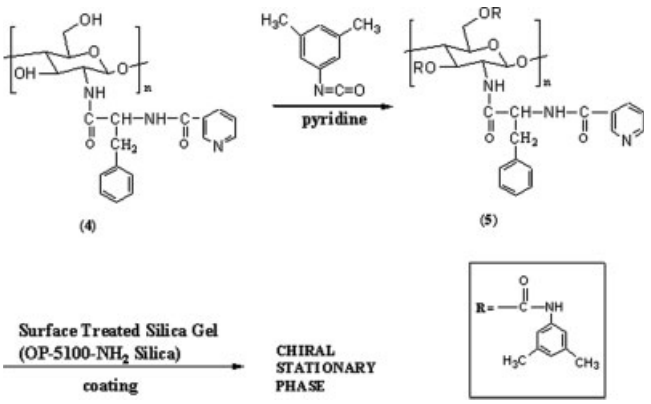
To a solution of trityl-chitosan 4.0 g (9.9 mmol) in *N*,*N*-dimethylacetamide 200 mL were added a solution of *N*-nicotinoyl-L-phenylalanine 2.68 g (9.9 mmol), HOBt · H_2O 1.52 g (9.9 mmol), and EDC · HCl 2.18 g (11 mmol) in dichloromethane 80 mL. The mixture was stirred at room temperature for 24 h. It was then cooled in an ice-water bath and 200 mL of deionized water was added. The precipitant was collected on a filter, washed with acetone and methanol, and vacuum dried to give a pale tan product (**3**) of 5.2 g (yield: 78.53%). To 2.90 g (4.32 mmol) of *N*-(*N*-nicotinoyl-L-phenylalanyl)-6-*o*-trityl chitosan was added 5 mL of dichloroacetic acid, and the mixture was stirred at room temperature for 2 h. The mixture was then poured into water to precipitate the product. It was filtered, washed with methanol and vacuum dried to give a pale tan product (**4**) of 1.5 g (yield: 80.9%); IR (KBr, 3): 3400 (amide), 3090 (arom), 2900 (cyclic alkane), 1650 (amide) cm^{-1}. IR (KBr, 4): 3424 (amide), 3065 (arom), 2927 (cyclic alkane), 1646 (amide), 1541 (amide) cm^{-1}.

1-isocyanato-3,5-dimethylbenzene of chitosan derivative(5) (Scheme 3)

Vacuum dried chitosan derivative (**4**) of 0.68 g (1.65 mmol) was dissolved into pyridine 15 mL with stirring at 80°C for 1 h. Into the solution, 1.265 mL (7.5 fold-excess) of 1-isocyanato-3,5-dimethylbenzene was added under flowing nitrogen, and the mixture was stirred at 80°C for another 24 h. The mixture was then poured into methanol and the precipitant was filtered. It was washed completely by soxhlet's extraction with methanol, and dried under vacuum to give a pale gray product (**5**) of 0.67 g (yield: 57.6%); IR (KBr): 3293 (amide), 3031 (arom), 2919 (cyclic alkane), 1564 (amide) cm^{-1}.

Coating silica with chitosan derivatives

To prepare a tetrahydrofuran (THF) solution of chitosan derivatives, 0.75 g of chitosan derivatives was dissolved into 10 mL of THF at room temperature for about 1 h. A few drops of chitosan derivatives solution in THF were added carefully drop by drop into the 50 mL round bottom flask containing 3.0 g of microporous silica powder to coat the silica powder with the chitosan derivatives. The flask with a few drops of chitosan derivatives solution and silica powder was then tapped against the palm for several times to mix them together. After repeating this process for four or five times, the flask was then connected to the vacuum evaporation set up to remove the THF remained in the silica powder. Evaporation of THF was carried out for half an hour under vacuum. This kind of process from dropping the chitosan derivatives solution into the flask to evaporation of THF under vacuum was repeated until the chitosan derivatives solution was completely used up.



Scheme 3 Reaction scheme for the synthesis of 1-isocyanato-3,5-dimethylbenzene of chitosan derivative (5) and the preparation of chiral stationary phase.

Preparation of chiral column for the HPLC

Chiral column packing materials prepared by coating the chitosan derivatives on microporous silica gel (RS Tech, particle size 5 μm) were packed in a stainless steel tube [15 cm × 0.46 cm (i.d)] by using slurry packing methods. The plate number of column was measured as 7255 for benzene with hexane-2-propanol [90 : 10(v/v), 0.5 mL/min] as the eluent.

RESULTS AND DISCUSSION

Synthesis

It has been known that the materials, which can be used as a chiral stationary phase (CSP) of the high performance liquid chromatography (HPLC), should have chiral centers in its molecular chain for the formation of chiral conditions that could distinguish the optical isomers to be separated. On this basis, chitosan that contains five chiral carbons in its ring structure of the main chain was chosen in this study as a base material for the development of a new CSP. It was expected that the capability of chitosan for the recognition of chiral compounds in particular the series of amino acids could be improved by the introduction of *N*-nicotinoyl-L-phenylalanine and 3,5-dimethylphenylcarbamate into the chitosan.

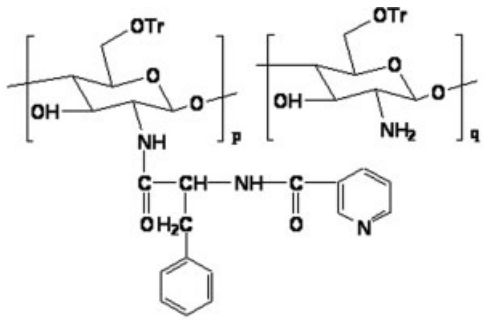


Figure 2 Degree of substitution calculated from the C/H/N of elemental analysis.

The details of the synthesis of the chitosan derivative containing the *N*-nicotinoyl-L-phenylalanine and 3,5-dimethylphenylcarbamate are as follows: *N*-nicotinoyl-L-phenylalanine(**1b**) was synthesized from the reaction of nicotinic acid with the active ester (**1a**) of *N*-hydroxysuccinimide (see Scheme 1). The synthesized *N*-nicotinoyl-L-phenylalanine was coupled with chitosan in the DMAc solution containing *N*,*N*′-dicyclohexylcarbodiimide (DCC) as a coupling reagent and *N*-hydroxylbenzotriazole (HOBt) as shown in Scheme 2 and Figure 2. The degree of substitution of the *N*-nicotinoyl-L-phenylalanine with the amine of the chitosan was determined to be 0.83 by the elemental analysis.

Before coupling the *N*-nicotinoyl-L-phenylalanine with chitosan, the chitosan was first reacted with phthalic anhydride to improve its solubility in organic solvents for the following reaction with TrCl in pyridine that was used as a protecting group of the OH— functional group of the chitosan. After introducing the Tr group to the OH— group of chitosan, the phthalic anhydride group was detached by the help of hydrazine to form the chitosan derivative containing the Tr group (2c). The 2c was then reacted with *N*-nicotinoyl-L-phenylalanine to introduce the *N*-nicotinoyl-L-phenylalanine group into the chitosan (3). The trityl protecting group was then removed from the chitosan by the reaction with dichloroacetic acid to form the chitosan derivative containing *N*-nicotinoyl-L-phenylalanine (4). The chitosan derivative with *N*-nicotinoyl-L-phenylalanine was then reacted with excess amount of 3,5-dimethylphenylisocyanate in the pyridine solution at room temperature for about a day to form the chitosan derivative with 3,5-dimethylphenylcarbamate and *N*-nicotinoyl-L-phenylalanine (5).

Characterizations

Molecular weights

The molecular weights of the modified chitosan containing 3,5-dimethylphenylcarbamate and *N*-nicotinoyl-L-phenylalanine (5) were determined using Gel Permeation Chromatography (GPC). As shown in Table I, the number average molecular weight (M_n) and the weight average molecular weight (M_w) of the modified chitosan were 17,166 and 28,100 g/mol, respectively, and its polydispersity was appeared to be about 1.64.

Solubility

Solubility of the modified chitosan (5) in various organic solvents were determined as shown in Table II. The solubility of the CSP is very important for the separation of materials by the HPLC, as well as for the formation of the chiral column using polymer-coating technique on the silica particles. When the material being used as a CSP is very soluble in all solution, it is impossible to use it without insolubilization process such as crosslinking. Also if that were insoluble in every solvent, it is very hard to make a chiral column using it.

It is well known that chitosan is an insoluble material in almost every organic solvent except in the ones with high acidity. So it is usually only soluble in acidic solution such as acidic aqueous solutions by transforming it into ionic structure. However, its solubility was improved in this study by modifying it with 3,5-dimethylphenylisocyanate and *N*-nicotinoyl-L-phenylalanine. The solubility of the polymer 4 containing *N*-nicotinoyl-L-phenylalanine group was improved more by the introduction of 3,5-dimethylphenylcarbamate into it to make polymer 5. The final product, polymer 5 with *N*-nicotinoyl-L-phenylalanine and 3,5-dimethylphenyl-carbamate showed good solubility in couple of organic solvents such as THF, pyridine and DMF. Replacing the hydroxyl group and amine group of chitosan with *N*-nicotinoyl-L-phenylalanine and 3,5-dimethylphenylcarbamate attributes the improved solubility of the polymer 5.

Thermal properties

Thermal stabilities of the CSP are important because sometimes wide range of operating temperature is

TABLE I
Molecular Weights of Polymer 5 Measured by GPC

M_n	M_w	MP	M_z	M_z+1	M_z/M_w	Polydispersity
17,166	28,100	28,490	39,843	50,846	1.4178	1.8369

TABLE II
Solubility of Polymer 5 in Various Solvents

	Toluene	Acetone	THF	Dichloro methane	Methanol	Water	Pyridine	DMSO	DMF
Polymer (4)	IS	IS	IS	IS	IS	IS	SS	S	VS
Polymer (5)	IS	S	VS	IS	IS	IS	VS	SS	S

VS, very soluble; S, soluble; SS, slightly soluble; IS, insoluble.

needed for the optimization of the operating conditions of the HPLC. For instance, when the T_g or T_m of the polymeric CSP is low enough to be about room temperature, the HPLC operating condition will be seriously limited.

To see the thermal properties such as T_g and T_m of the modified chitosans, polymers 4 and 5 were studied with DSC and TGA. From the DSC data, it was found that the T_g of the modified chitosans was not clear enough to be determined due to their rigid backbone structure, and T_m of them appeared to be close to their thermal degradation points. From the TGA data shown in Figure 3, it was found that the Polymer 5 that was used as a CSP in this study showed better thermal stability, compared with polymer 4. Especially, the degradation starting point of the polymer 5 (above 200°C) was much higher than that of the polymer 4. From this result, it was found that the polymer 5 is thermally stable enough to be used as a CSP for the HPLC column.

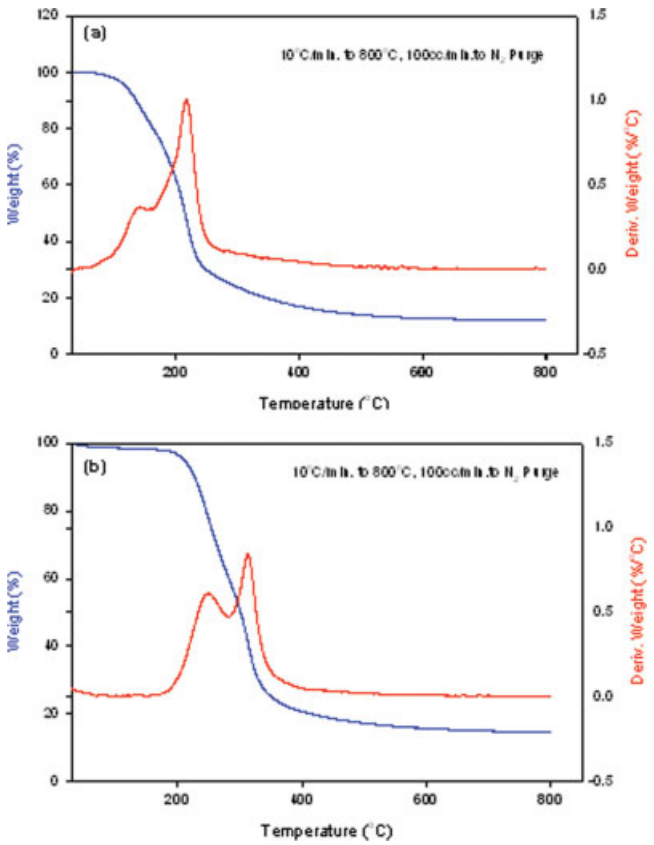


Figure 3 TGA curves of polymers **4** and **5**: (a) polymer **4**, (b) polymer **5**. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Morphological properties

The semicrystalline properties of the polymers 4 and 5 were studied with XRD. By transforming the chitosan to polymer 4, it became more crystalline (Crystallinity: 24.5%). Then the crystallinity of the polymer 4 disappeared as the polymer 4 was changed into polymer 5. Polymer 5 showed only slight amount of crystallinity (4.3%) as one can see in Figure 4.

Chromatographic enantioseparation

The polymer 5, modified chitosan with *N*-(*N*-nicotinoyl-L-phenylalanyl) and 3,5-dimethylphenylcarbamates groups, was used as a CSP for making a chiral column for the HPLC. THF soluble polymer *N*-(*N*-nicotinoyl-L-phenylalanyl)-chitosan bis(3,5-dimethylphenylcarbamates) (polymer 5) was coated on the surface of the silica particles as shown in Figure 5, showing good coating condition. By packing these silica particles into stainless column, using a slurry packing method, a chiral HPLC column was prepared and its separation performance was characterized.

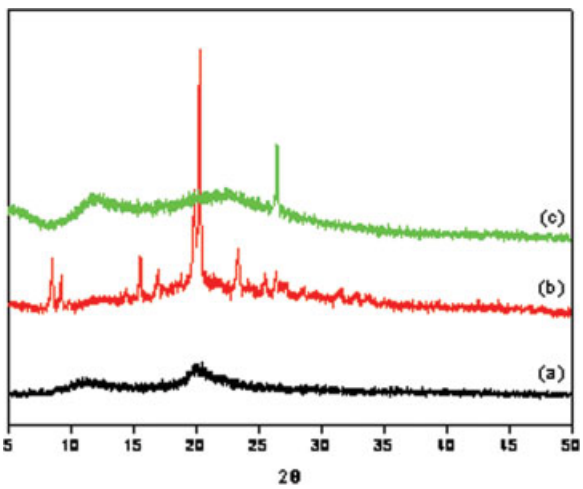


Figure 4 X-ray diffraction patterns of chitosan, polymers **4** and **5**: (a) chitosan, (b) polymer **4**, (c) polymer **5**. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

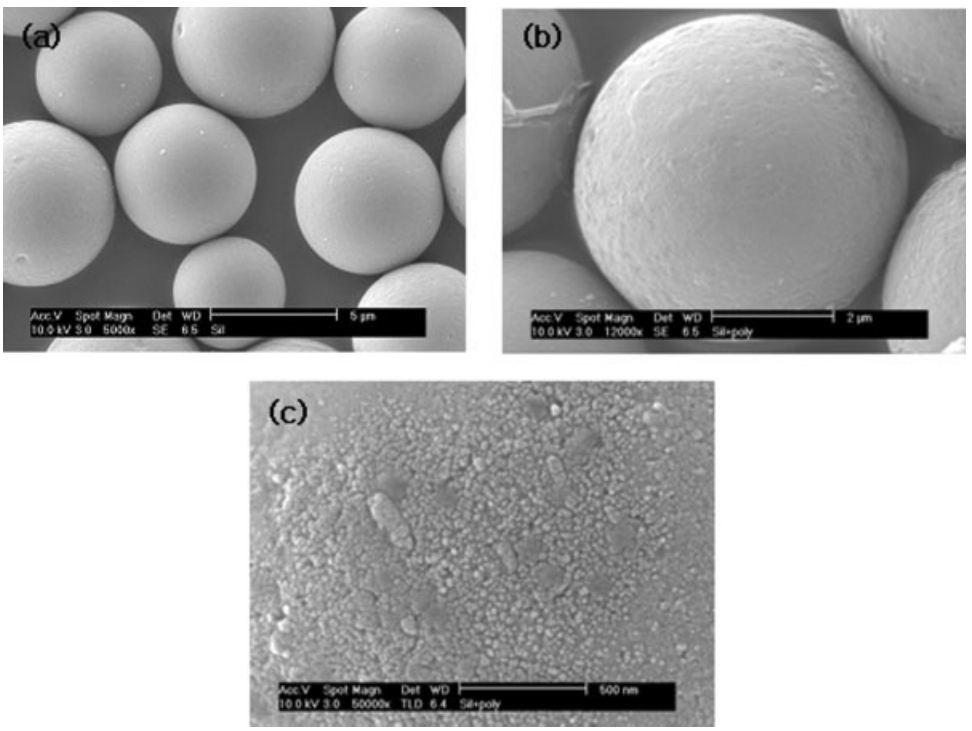


Figure 5 SEM photographs of silica particles before and after coating with the CSP based on chitosan; (a) before coating, (b) after coating, and (c) the magnification of the coated surface.

Table III shows the results of the separation of eleven standard racemic mixtures listed in Figure 1. The constants (k'_1, α, Rs) shown in Table III represent the details of the performance of the CSP based on polymer 5. The capacity factor (k'_1), determined by the retention times as shown in the eq. (1), indicates the degree of interaction between the CSP and the compound. So, the more the compound interacts with the CSP, the higher the value of k'_1.

$$k'_1 = (t_1 - t_0)/t_0 \quad (1)$$

where t_1 and t_0 are the retention times of the compound 1 and solvent, respectively.

The separation factor (α) determined by the capacity factors, k'_1 and k'_2, indicate how the CSP effectively separate the racemate. Generally speaking, when the separation factor is more than two, the separation is accepted as very good. On the basis of this separation factor, the CSP based on polymer 5 can be considered as good enough to be used practically for the separation of the optical isomers, since for most of the racemic compounds used in Table III, the separation factor is over two or very close to it.

$$\alpha = k'_2/k'_1 \quad (2)$$

where k'_2 and k'_1 represent the capacity factors of enantiomer 2 and enantiomer 1, respectively.

To be sure, the capability of the CSP for clear separation, the resolution (Rs) is used. The resolution factor represents how the two isomers are separated

TABLE III
Chromatographic Results

Racemic compound	CSP(5)				
	t_0(min)	k'_1 [a]	α	Rs	Mobile phase[b]
1	3.74	0.2	2.48	–	(a) 95 : 5
5	3.74	0.66	1.3	–	(a) 95 : 5
1	3.6	0.19	1.96	0.98	(a) 80 : 20
3	3.6	0.37	1.68	1.29	(a) 80 : 20
8	3.6	0.37	4.70	4.33	(a) 80 : 20
1	3.5	0.31	4.22	4.1	(a) 75 : 25
6	3.5	0.21	1.54	–	(a) 75 : 25
8	3.5	0.36	3.69	3.49	(a) 75 : 25
1	3.7	0.17	4.28	2.19	(a) 60 : 40
2	3.7	0.68	1.21	0.50	(a) 60 : 40
5	3.7	0.1	2.19	–	(a) 60 : 40
9	3.7	0.29	2.13	1.54	(a) 60 : 40
3	3.5	0.14	1.72	–	(b) 60 : 40 : 0.2
4	3.5	0.23	2.05	1.19	(b) 60 : 40 : 0.2
10	3.3	1.63	1.88	1.81	(c) 50 : 50
11	3.3	1.65	2.01	3.19	(c) 50 : 50
9	3.2	0.93	2.76	7.97	(c) 25 : 75
10	3.2	0.88	1.95	7.26	(c) 25 : 75
11	3.2	0.90	2.02	7.18	(c) 25 : 75

[a] k'_1, capacity factor for the first eluted enantiomer; α, selectivity factor; Rs, resolution. Column: 150 × 46 mm^2. Flow rate: 1 mL/min.

[b] (a) hexane/IPA; (b) hexane/IPA/TFA; (c) hexane/chloroform.

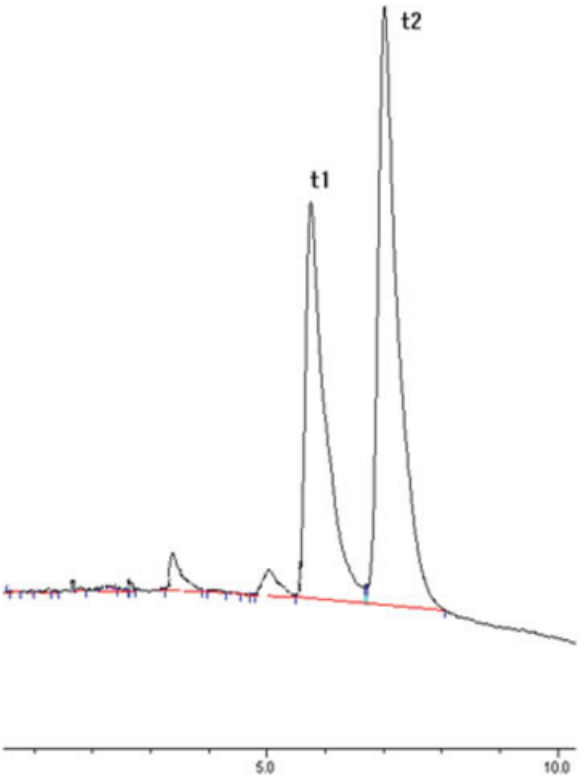


Figure 6 Chromatographic resolution of pindolol (**11**) [Mobile phase: hexane/chloroform (25 : 75)]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

without dragging effect by the CSP. So, when the value of Rs is over one, it is accepted that the two isomers are separated without overlapping. The Rs is determined using the following eq. (3).

$$\mathrm{Rs} = 2\ \Delta t/(W_2 + W_1) \tag{3}$$

where Δt is the retention time difference between peaks for isomers 1 and 2, and W_2 and W_1 are the widths of the peaks 1 and 2. The resolution factors for most of the racemates used in this study appeared to be over one, for some compounds over seven. From these results, it can be concluded that with proper composition of the moving phases used, the column prepared by using the CSP base on polymer 5 is good enough to be used for the separation of the most of the enantiomers used.

Figure 6 shows the chromatographic separation result of the racemic pindolol. Two peaks representing each enantiomer appeared separated at t_1 and t_2 showing good separation performance of the column. This kind of good performance of the polymer 5 as a CSP for the separation of enantiomers can be explained by the same theory discussed in the previous reports by Okamoto et al. They said in the papers about the cellulose phenylcarbamate ester derivatives CSP that the H-bonding interactions, π–π interaction and dipole–dipole interactions exerting between cellulose derivatives used as a CSP and racemic compound to be separated are important factors for the their separation.[17,18] It is believed that the introduction of *N*-nicotinoyl-L-phenylalanine group into the chitosan containing 3,5-dimethylphenylcarbamate increased the number of sites that can interact with racemic mixtures, improving its separation performance.

CONCLUSIONS

The chitosan derivative with *N*-nicotinoyl-L-phenylalanine group and 3,5-dimethylphenylcarbamate group can be prepared in good yield by the modification reaction of chitosan. It was very soluble in several organic solvents and therefore easy to coat silica particle by the solution coating method to make chiral column for the HPLC. Its thermal property was good enough to be used as a CSP up to over 100°C of operating temperature. Its chiral separation performance turned out to be good.

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