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# CORRESPONDENCE

## Derivatized Cyclodextrins Immobilized on Fused-Silica Capillaries for Enantiomeric Separations via Capillary Electrophoresis, Gas Chromatography, or Supercritical Fluid Chromatography

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### INTRODUCTION

A number of different derivatized cyclodextrins have been used as stationary-phase coatings for capillary gas–liquid chromatography (GLC) in the last few years.[1-7] Two different approaches have been used. The first involved extensive derivatization of the cyclodextrin with one or more hydrophobic and/or moderately polar substituents in order to obtain a gelatinous or amorphous material. This material actually consisted of a mixture of homologues and isomers and could be coated directly on the inside wall of the capillary.[1,2,5-7] The second approach consisted of taking simple methoxy-functionalized cyclodextrins and dissolving them in an appropriate GC liquid stationary phase (e.g., polysiloxane, polyethylene glycol, etc.)[3,4] In general, the first type ("liquified cyclodextrin derivatives") separated a greater variety of compounds and with shorter columns than the "dissolved-cyclodextrin stationary phases".

Both types of cyclodextrin-based GLC stationary phases are physically similar in that they are coated and not immobilized on the capillary wall. When coated columns are inadvertently subjected to extremes of temperature, flow, large injection volumes, etc., the integrity of the coating may change and the efficiency can decline. Also, coated capillary columns generally have shorter useful lifetimes when used with supercritical fluids or in solvent-based techniques such as capillary electrophoresis and open tubular liquid chromatography.

In this work, we report a relatively simple immobilized cyclodextrin-based stationary phase for fused-silica capillaries. This stationary phase appears to be stable and relatively unchanged by high temperature. Furthermore, it cannot be removed or stripped from the capillary wall by conventional polar and nonpolar solvents used in electrophoresis and liquid chromatography or by supercritical fluids. The successful use of this "derivatized-cyclodextrin capillary" in capillary electrophoresis, gas chromatography, and supercritical fluid chromatography is demonstrated.

### EXPERIMENTAL SECTION

**Materials.** Native β-CD was obtained from Advanced Separations Technologies (Whippany, NJ). Untreated fused-silica capillaries were obtained from Supelco (Bellefonte, PA). Commercial pretreated capillaries were purchased from Restek (Bellefonte, PA). Microbore fused-silica capillaries for capillary electrophoresis (CE) and super fluid chromatography (SFC) were purchased from P. J. Cobert Association (St. Louis, MO). Azo-*tert*-butane (ATB) was purchased from Lancaster (Windham, NH) and hydrogen-terminated poly(dimethylsiloxane) of 400 average molecular weight (PS537) and poly(methylhydrosiloxane) of 4500–5000 average molecular weight (PS122) were from Huls (Bristal, PA). Carbowax 550 was purchased from Ohio Valley Specialty Chemical (Marietta, OH). All other materials were obtained from Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), or Fluka (Ronkonkoma, NJ).

**Methods. A. Synthesis of Stationary Phase.** As shown in Figure 1, the native β-cyclodextrin (I) was first converted into the unsaturated derivative (II) and then permethylated (III). Compound III could be coupled with organohydrosiloxane polymer, yielding a yellowish, viscous stationary phase (IV). Two representative reaction procedures are given below.

**Synthesis of Permethylated Allyl-Substituted β-Cyclodextrin.** The procedure described in this section was carried out in a glovebox (neoprene gloves) containing a positive pressure of dry nitrogen gas. The glovebox was vented to a hood. β-Cyclodextrin (β-CD; 6.76 g, 6 mmol) was dried in vacuo at 100 °C over $P_2O_5$ for 8–10 h. The dried β-CD was dissolved in 200 mL of anhydrous dimethyl sulfoxide (DMSO) in a 500-mL, three-necked, round-bottom flask fitted with a thermometer and a dropping funnel. After dissolution was complete, 3.36 g (84 mmol) of powdered NaOH was added to the cyclodextrin solution. The mixture was stirred with a magnetic stirrer at room temperature for 4–5 h. A viscous, orange solution was produced. Approximately 2.6 mL (30 mmol) of allyl bromide (Figure 1) was dissolved in 10 mL of dry DMSO. This solution was placed in a dropping funnel and dispensed to the cyclodextrin solution at a rate of 40 drops/min. The permethylation reaction was carried out by adding 3.03 g (126 mmol) of sodium hydride crystals to the solution containing the allyl-substituted CD. Caution must be exercised as hydrogen gas was vigorously released and the solution temperature increased to about 35 °C. After the solution was stirred for 1 h, 6.0 mL (96 mmol) of methyl iodide mixed with 20 mL of dry DMSO was added dropwise to the derivatized β-CD solution. This mixture was allowed to stir for 16–24 h in order to achieve complete reaction. The flask was then removed from the glovebox, and the solution was poured into 500 mL of $H_2O$. The aqueous mixture was extracted three times with 50-mL portions of chloroform. The three chloroform layers were collected and washed three times with 500 mL of water. The final chloroform solution was dried overnight over anhydrous sodium sulfate. After the solid $Na_2SO_4$ was filtered, the solvent was removed with a rotory evaporator and the solid compound was recrystallized from a mixture of 30 mL of chloroform and 30 mL of hexane. The final product was wrapped with aluminum foil for future use. The presence of unsaturation in this product was checked by aqueous bromine addition to the double bonds and by observation of the appropriate absorption band in the infrared spectrum. The yield was approximately 75%. The other

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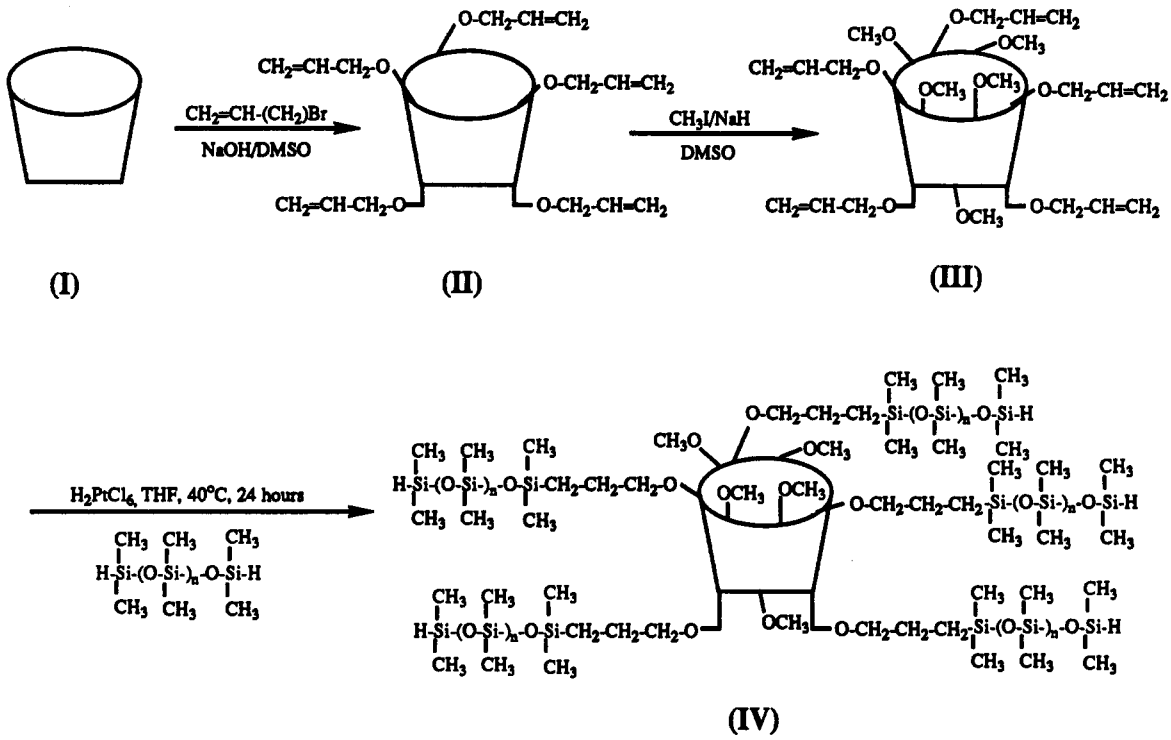


**Figure 1.** Synthesis of the derivatized β-cyclodextrin and organohydrosiloxane copolymer. β-Cyclodextrin has 14 secondary hydroxyl groups at the wide end of the torus and 7 primary hydroxyls at the opposite end. For the purpose of simplicity, only a few groups are shown in this schematic. After the first synthetic step there are approximately 5 allyl (or pentenyl) substituents on each β-cyclodextrin molecule.

**Table I. Stability Parameters for Three Immobilized β-CD Stationary Phases**

| stationary-phase ratio of β-CD derivative to siloxane oligomer | ATB vapor purge conditions | | | curing conditions | | solvent dissolution params[a] | | |
|---|---|---|---|---|---|---|---|---|
| | $N_2$ flow rate (mL/min) | temp (°C) | h | temp (°C) | h | $Z_k$ (%) | $Z_n$ (%) | $Z_\alpha$ (%) |
| 1:4 5-APβCD:PS537[c] | 0.60 | 23 | 0.67 | 30–150 at 1 °C/min | 15.0 | 109 | 92 | 102 |
| 1:6 5-APβCD:PS537[d] | 0.05 | 30–150 at 1 °C/min | 23.0 | 150 | 11.0 | 97 | 95 | 98[b] |
| 1:6 7-PPβCD:PS537[d] | 0.15 | same | 3.0 | 150 | 8.5 | 100 | 90 | 100 |

[a] All parameters ($Z_k$, $Z_n$, $Z_\alpha$) are given in the Experimental Section. [b] The enantiomeric separation test compound for this column was ethyl lactate. [c] Home-pretreated fused-silica tubing. [d] Polar commercial pretreated fused-silica tubing.

derivatized β-CD (i.e., when 5-bromo-1-pentene was used instead of allyl bromide in Figure 1) was synthesized in analogous manner.

**B. Coupling Derivatized β-Cyclodextrin to the Organohydrosiloxane Polymer.** Approximately 0.25 g of permethylated allyl-substituted β-CD (or the pentenyl-derivatized β-CD) was dried in vacuo at 100 °C over $P_2O_5$ for 8 h. The desired amount of PS 537 was placed in a 25-mL, three-necked, round-bottom flask equipped with a thermometer and a condenser. After the mixture was dissolved in 10 mL of tetrahydrofuran (THF), the reaction was activated by adding 25 μL of 10% platinic chloride solution in THF. The reaction was stirred at 40 °C for 24 h. The solution was then transferred into a glass vial, and the solvent was evaporated by placing the vial on the surface of hot plate at ~50 °C. After the evaporation was completed (in ~20 h), the stationary-phase material consisted of a yellowish, viscous liquid and was stored in a refrigerator for future use. Prior to the evaporation step the THF should be checked for peroxides. Only small amounts of reagent were used in this step.

**C. Pretreatment of Fused-Silica Capillaries.** Approximately 90% of a 20 m × 0.25 mm i.d. fused-silica capillary column was filled with 18% (w/w) hydrochloric acid. The "both-end-sealed" capillary was heated at 90 °C for 30 min. The column was then washed with four column volumes of $H_2O$. Approximately 90% of the column was subsequently filled with 5% (w/w) aqueous sodium hydroxide solution and then cured at 125 °C for 30 min. The column was washed with four column volumes of $H_2O$ and acetone and then flushed with dry nitrogen for 30 min. The column was then statically coated with a 0.04% methylene chloride solution of Carbowax 550 and PS 122 (50:50, w/w) by a normal static coating procedure.[5-7] After coating the column was flushed with nitrogen for 30 min, both ends were flame-sealed, and the column was placed in a 290 °C oven for 2 h. Deactivation was done by thermal curing mechanism.[9,10]

**D. Preparation of Open-Tubular Capillaries.** Columns for gas chromatography (GC) use were prepared as follows. A pretreated column (10 m × 0.250 mm i.d.) was coated using the static method and a filtered 0.4% ethyl ether solution of the synthesized stationary phase. After the coated column was flushed with dry nitrogen gas for ~30 min, the column was conditioned from 35 to 150 °C at the rate of 2 °C/min. A conditioned column was then tested with naphthalene at 90 °C for column efficiency and α-ionone at 120 °C or 1,5,8-trimethyltetraline at 100 °C for enantiomeric selectivity. The cross-linking process was initiated afterward. Columns for CE and SFC were 1 m × 0.05 mm i.d. and 4 m × 0.05 mm i.d. fused-silica capillaries, respectively. They were statically coated with a 2% diethyl ether solution of the derivatized cyclodextrin.

**E. Immobilization of Stationary Phase.** Coated columns were purged with Azo-*tert*-butane vapor in nitrogen for 40 min to 3 h at a very low nitrogen flow rate. This step was done in

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**Table II. Thermal Stability Test Results[a]**

| | stationary phase | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | immobilized 1:6 7PPβCD:PS537[b] | | | immobilized 1:4 5APβCD:PS537[b] | | | coated 1:4, 5APβCD:PS537[b] | | |
| temp (°C) | $d_k$ | $d_n$ | $d_\alpha$ | $d_k$ | $d_n$ | $d_\alpha$ | $d_k$ | $d_n$ | $d_\alpha$ |
| 150 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 170 | 100 | 80 | 100 | 112 | 90 | 101 | 108 | 50 | 99 |
| 190 | 110 | 60 | 99 | 130 | 75 | 101 | 130 | 9 | 100 |
| 230 | 113 | 40 | 99 | 137 | 50 | 100 | 165 | 1 | 101 |
| 250 | 113 | 30 | 99 | 139 | 40 | 100 | 170 | 0.7 | 99 |

[a] All parameter definitions ($d_k$, $d_n$, $d_\alpha$) are given in the Experimental Section. [b] Polar commercial pretreated fused-silica tubing.

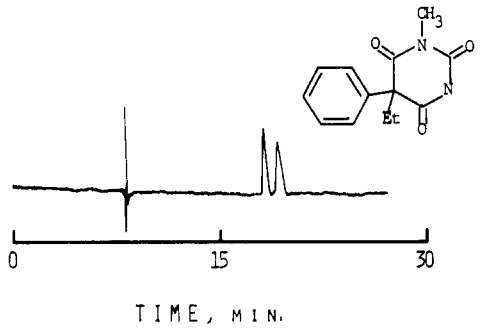


**Figure 2.** Capillary electrophoresis resolution of racemic mephobarbitol on a 50 μm i.d. × 65 cm long fused-silica capillary with wall-immobilized, derivatized β-cyclodextrin. The mobile phase consisted of pH 7.8, 50 mM phosphate buffer. The separation was run at 20 kV and 60 μA. The distance from injector to the detector was 40 cm. The UV detection wavelength was 254 nm. Approximately 1 nL of 1 mg/mL racemic mephobarbitol standard was injected. See the Experimental Section for further details.

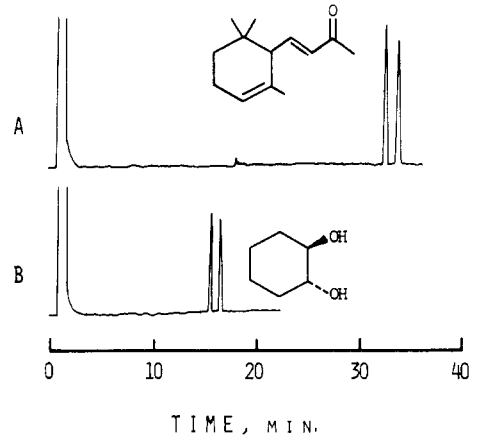


**Figure 3.** (A) GC separation of enantiomers of α-ionone on a 250 μm i.d. × 10 m long fused-silica capillary with wall-immobilized, 1:4, allylpermethyl-β-CD:PS537 (i.e., the first column in Table I). The column temperature was 120 °C. Approximately 0.05 μL of 1 mg/mL α-ionone solution (in ether) was injected. (B) GC separation of enantiomers of *trans*-1,2-cyclohexanediol. All experimental conditions were identical to those of chromatogram A except that a programmed temperature gradient was used (100–150 °C at 2 °C/min). See the Experimental Section for further details.

a hood. Flame-sealed columns were then heated from 30 to 150 °C at the rate of 2 °C/min and held at 150 °C for 9–12 h. The cross-linked column was washed with 2–3 mL of ethyl ether at the rate of 1 mL/h. The column was then flushed with dry nitrogen gas for 2 h and attached to a gas chromatograph to reevaluate column efficiency, $k'$ values, and chiral selectivity. The degree of cross-linking was calculated from the relationship $Z_k = (k'_2/k'_1) \times 100\ (\%)$, where $k'_1$ and $k'_2$ are the capacity factors of naphthalene at 90 °C before and after washing the column. The relative efficiency was calculated from the relationship $Z_n = (n'/n) \times 100\ (\%)$, where $n$ and $n'$ are the column efficiencies (number of theoretical plates) for naphthalene at 90 °C before and after washing. The relative enantiomeric separation factor was calculated from the relationship $Z_\alpha = (\alpha'/\alpha) \times 100\ (\%)$, where $\alpha$ and $\alpha'$ are the enantiomeric separation factors for α-ionone at 120 °C before and after washing. Table I lists stability parameters for three different immobilized β-cyclodextrin stationary phases. An analogous experiment with the equivalent, but not immobilized stationary phase showed that all of the coating was stripped from the capillary wall. Immobilization on the column for SFC was done by heating the column from 30 to 150 °C at the rate of 1 °C/min and keeping the column at the upper temperature for 10 h while a trace amount of $N_2$ was purged through the column. After the column was rinsed with ether, the differences in capacity factor and chiral selectivity were found to be negligible.

**F. Procedure for the GC Thermal Stability Test.** Columns selected for thermal stability evaluation were first characterized and then conditioned from 50 to 170 °C at the rate of 5 °C/min and held at the upper temperature for 12 h. After thermal conditioning, the columns were tested for changes in efficiency and retention (with naphthalene at 90 °C) as well as chiral resolving power (with α-ionone at 120 °C). The reltive difference in the capacity factor was calculated from the relationship $d_k = (k'_2/k'_1) \times 100\ (\%)$, where $k'_1$ and $k'_2$ are the capacity factors before and after conditioning. Relative differences in column efficiencies were calculated from the relationship $d_n = (n_2/n_1) \times 100\ (\%)$, where $n_1$ and $n_2$ are the column efficiencies (number of theoretical plates) before and after conditioning. The relative differences in enantiomeric separation factors were calculated from the relationship $d_\alpha = (\alpha_2/\alpha_1) \times 100\ (\%)$, where $\alpha_1$ and $\alpha_2$ are the enantiomeric separation factors before and after conditioning. The columns were then conditioned in the same manner at a temperature 20–30 °C higher. This stepwise conditioning and evaluation was continued to 275 °C. Table II compares the thermal stability of two immobilized stationary phases with the analogous coated phase.

**G. Apparatus and Methods.** A Varian Model 3700 gas chromatograph equipped with a flame ionization detector was used for all GC evaluations and separations. Approximately 0.05–0.1 μL of sample was injected using a split ratio of 1:100. The injection port and detector temperature were set at 250 °C. Nitrogen was used as the carrier gas with a linear velocity of ~10 cm/s (gas pressure inlet of ~2.8 psi). Capillary electrophoresis was done on an Isco 3850 electropherograph. The exact experimental conditions are given in the appropriate figure legend. Supercritical fluid separations were done on a Hewlett-Packard Model G1205A unit. Further experimental conditions are given in the related figure legend.

## RESULTS AND DISCUSSION

In order to be useful for a variety of capillary-based separation methods, an immobilized cyclodextrin phase must meet certain requirements for both stability and selectivity. Tables I and II (Experimental Section) summarize solvent and thermal stability results, respectively, for typical immobilized cyclodextrin–polysiloxane stationary phases. Washing a coated but not immobilized fused-silica capillary column with methylene chloride, diethyl ether, or hydroorganic solvents results in the removal of the cyclodextrin-based stationary phase. However, after immobilization the capillary can be thoroughly rinsed with a variety of solvents without significantly affecting retention ($k'$), efficiency ($n$), or enantiomeric selectivity ($\alpha$) (see Table I). Generally less than 3% of the stationary phase is lost after repeated washings. This

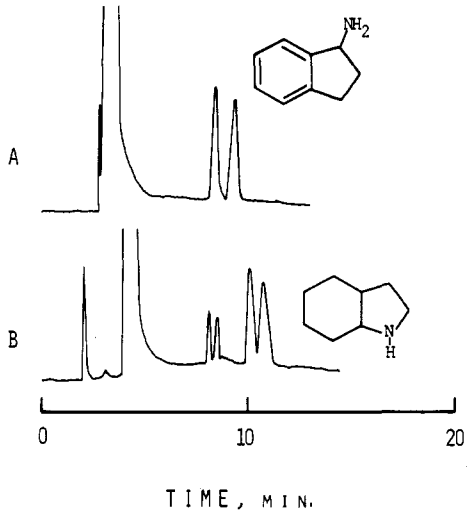


**Figure 4.** (A) SFC resolution of 1-aminoindan on a derivatized β-cyclodextrin immobilized fused-silica capillary. This capillary was identical to that used in CE (Figure 2) exept that it was 4 m long. The separation was done at 60 °C using unmodified $CO_2$ as the mobile phase. The density gradient was programmed at 0.01 g/(mL·min$^{-1}$) after holding for 1 min at an initial density of 0.35 g/mL after the injection. One microliter of 0.2% (v/v) analyte solution (in methylene chloride) was injected. See the Experimental Section for further details. (B) SFC resolution of both pairs of enantiomers of perhydroindole. Experimental conditions were identical to those described for chromatogram A above except that density gradient was programmed at 0.004 g/(mL min$^{-1}$).

is significantly less than other approaches.[8] As expected, immobilization also gives the stationary phase greater thermal stability than the coated analogue (Table II). The exceptional stability of these stationary phases may be the result of the high degree of branching and cross-linking of the cyclodextrin–siloxane copolymer.

Immobilized cyclodextrin-based capillaries can be used for both chiral and achiral separations in capillary electrophoresis (CE), gas chromatography (GC), and supercritical fluid chromatography (SFC). Figure 2 is a capillary electropherogram showing the baseline resolution of the racemic barbiturate, mephobarbitol. As can be seen the solvent consisted only of aqueous buffer (i.e., no chiral mobile-phase additives). In addition, no gel or packing material was contained in the capillary. The separation was very reproducible and required little experimental preparation other than adjusting the pH and concentration of the buffer.

Figure 3 shows the gas chromatographic resolution of racemic α-ionone and *trans*-1,2-cyclohexanediol on the immobilized derivatized β-CD capillary. Many of the compounds resolved on this capillary are not easily separated on any other known coated GC chiral stationary phase. Interestingly, most chiral diols must be made into their trifluoroacetyl derivatives before they can be resolved on coated columns.[6,7] This is done to increase their volatility as well as enantioselectivity. However, all of these chiral diols can now be resolved at higher temperatures, without derivatization by using the immobilized-cyclodextrin capillary (Figure 3).

Figure 4 shows the supercritical fluid chromatographic resolution of all stereoisomers of 1-aminoindan and perhydroindole. Note that perhydroindole consists of two pairs of enantiomers [the (±) cis and (±) trans]. Also, it should be noted that the capillaries used in SFC subsequently have been used successfully in CE. However, for routine use, SFC usually requires a longer capillary column than CE. Currently, the overall applicability of the wall-immobilized cyclodextrin capillaries is being evaluated by CE, GC, and SFC. It is expected that subsequent reports will document the resolution of several hundred enantiomeric pairs via these capillary techniques.

## CONCLUSIONS

Clearly capillary columns containing immobilized cyclodextrin coatings will be useful for a variety of separation methods. Although hardy, they will not completely replace the coated columns for GC separations. This is because the enantioselectivity of the bonded column is somewhat different from many of the coated columns. Also there is a greater variety of coated columns, which means a greater variety of enantiomers and other compounds can be resolved. Finally, for lower temperature GC separations, the performance and enantioselectivity of the coated columns sometimes exceeds that of the bonded-phase columns. However for longevity and performance at high temperatures, with supercritical fluids, or in the presence of solvents, the immobilized cyclodextrin-based stationary phase is not only effective, but often essential.

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