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Preparative Enantiomer Separation of Dichlorprop with a Cinchona-Derived Chiral Selector Employing Centrifugal Partition Chromatography and High-Performance Liquid Chromatography: A Comparative Study

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A countercurrent chromatography protocol for support-free preparative enantiomer separation of the herbicidal agent 2-(2,4-dichlorphenoxy)propionic acid (dichlorprop) was developed utilizing a purposefully designed, highly enantioselective chiral stationary-phase additive (CSPA) derived from bis-1,4-(dihydroquinidinyl)phthalazine. Guided by liquid−liquid extraction experiments, a solvent system consisting of 10 mM CSPA in methyl *tert*-butyl ether and 100 mM sodium phosphate buffer (pH 8.0) was identified as a suitable stationary/mobile-phase combination. This solvent system provided an ideal compromise among stationary-phase retention, enantioselectivity, and well-balanced analyte distribution behavior. Using a commercial centrifugal partition chromatography instrument, complete enantiomer separations of up to 366 mg of racemic dichlorprop could be achieved, corresponding to a sample load being equivalent to the molar amount of CSPA employed. Comparison of the preparative performance characteristics of the CPC protocol with that of a HPLC separation using a silica-supported bis-1,4-(dihydroquinidinyl)phthalazine chiral stationary phase CSP revealed comparable loading capacities for both techniques but a significantly lower solvent consumption for CPC. With respect to productivity, HPLC was found to be superior, mainly due to inherent flow rate restrictions of the CPC instrument. Given that further progress in instrumental design and engineering of dedicated, highly enantioselective CSPAs can be achieved, CPC may offer a viable alternative to CSP-based HPLC for preparative-scale enantiomer separation.

Chromatographic separation of racemic mixtures on chiral stationary phases (CSPs) is recognized as an efficient approach to access scaleable amounts of pure enantiomers.[1] Its rapid maturation from a purely analytical to a widely accepted industrial production tool was facilitated by major progress achieved in chromatography process engineering[2] and, in particular, enantioselective adsorbent development.[3] The systematic evolution of enantioselective receptors provided a rich toolbox of chiral selectors (CSs), capable of resolving virtually any racemic mixture of interest. Developing dedicated immobilization strategies to confine these CSs onto the surface of appropriate support materials was a key for the generation of robust bonded CSPs, demonstrating long-term stability under continuous operation conditions.

Nevertheless, there are also inherent limitations associated with preparative applications of bonded CSPs. CSP preparation requires expensive support materials fulfilling strict criteria in terms of chemical inertness, particle size and shape, controlled porosity and surface properties. Also, CS immobilization often involves sophisticated and cost-intensive chemistries. Additional efforts have to be invested into column packing, a nontrivial task at technical scale.[4] Drawbacks may arise from the chemical microenvironment in which the CS units are embedded in bonded CSPs. The physical constraints imposed by the attachment may compromise CS accessibility and induced fit-type chiral recognition phenomena.[5] Further, the inevitable introduction of additional chemical entities (residual surface functionalities, spacer and linker groups) may give rise to competing nonspecific interactions, which interfere with stereoselective association processes.[6−8] A serious restriction of bonded CSPs are the notoriously low CS densities that can be generated on the limited surface areas (e.g., for silica gels <350 m^2/g) of conventional support matrixes, resulting in relatively low preparative capacities.[1] Depending on the size and shape of the CSs, and the immobilization chemistry used for grafting, the supports permit surface loading levels in

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the modest range of 0.3−1.0 μmol of CS/m^2. Higher CS loading may be achieved with polymer coating technology,[5] but at the risk of compromised CS accessibility and function due to steric overcrowding. Another problematic issue in context with the use of bonded CSPs at preparative scale concerns irreversible adsorption of contaminants.[2,9] This may alter the overall adsorption characteristics of bonded CSPs, enforcing reoptimization of operation parameters, elaborate washing protocols, or ultimately a complete exchange of the affected CSP material.

Many of the problems inherent to bonded CSPs may be resolved by resorting to alternative preparative chromatographic methodologies that completely circumvent CS immobilization. Specifically, support-free liquid−liquid partition chromatographic technologies, e.g., countercurrent chromatography (CCC) and the conceptually closely related centrifugal partition chromatography (CPC), may provide such alternatives.[10,11] These techniques use immiscible solvents (or solvent mixtures) as stationary and mobile phases. During the chromatographic process, the liquid stationary phase is "immobilized" in the column compartment by a strong gravitational field, generated by centrifugation, while the mobile phase is forced to percolate the former by pumping. To create an enantioselective stationary phase, a suitable CS is dissolved in an appropriate solvent, acting as a chiral stationary-phase additive (CSPA). The separation of the enantiomers introduced with the mobile phase is then effected by selective partition due to the differential stabilities of the diastereomeric CSPA−analyte complexes formed in the stationary phase.

The use of stationary phases with physically unconfined CS units may offer various practical advantages over the immobilized CS regime in bonded CSPs. Most appealing, the process of preparing an enantioselective stationary phase is simplified to dissolving the CSPA in an appropriate solvent, suspending any need for expensive solid supports and sophisticated immobilization chemistries. Column packing can be achieved with ease by filling the column compartment with the CSPA solution by pumping. Using CSs as free solution species also obviates any support-induced conformational restrictions and nonspecific interaction interfering with selective CS−analyte association. The CS loading level (and thus the preparative capacity) of the stationary phase may conveniently be adjusted over a broad concentration range, with limits being theoretically dictated only by CSPA solubility.

Attracted by these potential benefits, several research groups have studied the utility of CCC and CPC for enantiomer separations. The results of these investigations have been discussed in detail in recent review articles.[11] Various CS systems, including bovine serum albumin,[12−14] π-acidic amino acid derivatives,[15−18] sulfated β-cyclodextrins,[19] the antibiotic vancomycin,[20] and cinchona alkaloid derivatives,[21] have been successfully employed for enantiomer separation of amino acid derivatives, drugs, and metabolites. Preparative runs performed in the course of these studies gave promising results,[16,19−21] encouraging further research in this field.

However, a problem currently limiting routine application of CPC/CCC for enantiomer separation is the lack of variety of efficient CSPAs. CSs developed for bonded CSPs frequently fail in CPC/CCC application due to insufficient enantioselectivity, unfavorable solubility and phase distribution characteristics, and incompatibility of the molecular recognition mechanisms with mobile- or stationary-phase solvents.[11,20] Specific chemical modification of existing highly enantioselective CS systems, however, may allow generation of dedicated CSPAs fulfilling the multifaceted criteria in terms of enantioselectivity, solubility, and phase-transfer properties.[21]

Addressing these issues, we report here on the development of preparative CPC and HPLC enantiomer separation protocols for dichlorprop utilizing a dedicated CSPA and a bonded CSP, respectively, both derived from bis-1,4-(dihydroquinidinyl)phthalazine (Figure 1). We outline important aspects concerning design and synthesis of the CSPA and discuss the systematic optimization of the operation conditions of the chromatographic separation protocol. The preparative performance characteristics of these complementary enantiomer separation methodologies are assessed and critically evaluated on the basis of productivity-related criteria and environmental considerations.

EXPERIMENTAL SECTION

General Information. Unless stated otherwise, all reactions were carried out under strictly anhydrous conditions and under nitrogen atmosphere. All solvents were dried according to standard procedures and distilled prior to use. The ^{1}H NMR spectra were acquired on a Bruker DRX 400-MHz spectrometer. The chemical shifts (δ) are given in parts per million (ppm) relative to TMS as internal standard. IR spectra were recorded with a Perkin-Elmer Spectrum 2000 spectrometer. Mass spectra were acquired on a PESciex API 365 triple quadrupole instrument using electrospray ionization. Sample solutions in appropriate solvents (chloroform/methanol) were infused at concentrations of ~0.1 mg/mL via a syringe pump at a flow rate of 5 μL/min. The electrospray voltage was typically set to 5250 V. Optical rotation values were measured on a Perkin-Elmer 341 polarimeter at 25 °C. Melting points were determined with a Kofler apparatus, equipped with a Leica Galen III microscope. Thin-layer chromatography was carried out with Silica gel 60 F_{254} aluminum sheets provided by Merck (Darmstadt, Germany). Flash chromatography was performed on Silica 60 (0.040−0.063-mm particle size (Merck).

Materials. 2-(2,4-Dichlorophenoxy)propionic acid (dichlorprop), butyllithium, 1,4-dichlorophthalazine, tetrahydrofuran (THF), potassium carbonate, potassium hydroxide, and octadecylmer-

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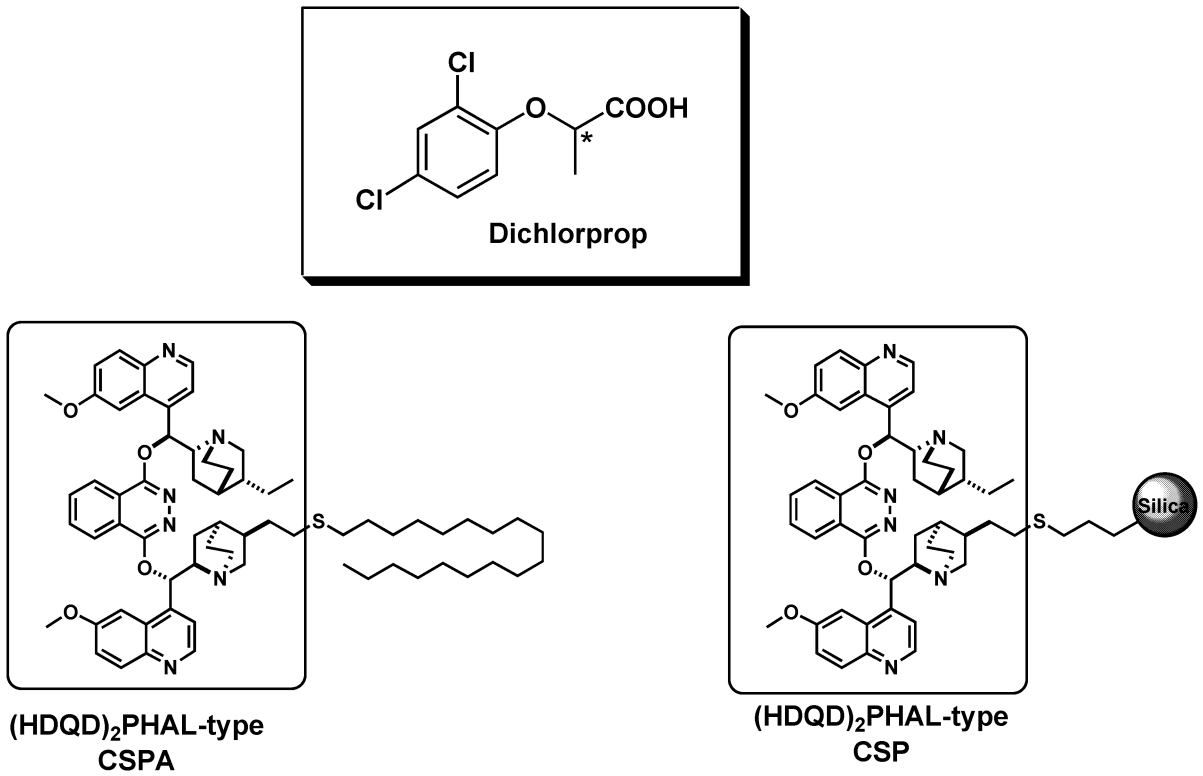

Figure 1. Structures of the chiral herbicidal agent dichlorprop and the co-generic CSP and CSPA derived from $(DHQD)_2PHAL$.

captan were purchased from Aldrich (Vienna, Austria). Dihydroquinidine (DHQD) was provided by Boehringer Mannheim (Mannheim, Germany) and quinidine (QD) was bought from Buchler (Braunschweig, Germany). Ammonium acetate, acetic acid, and methyl *tert*-butyl ether (MTBE) were purchased from Fluka (Buchs, Switzerland). α,α'-Azoisobutyronitrile was from Merck. Magnesium sulfate was purchased from Riedel de Haen (Vienna, Austria). Spherical silica gel (ProntoSIL 120-5-Si, 5 μm, 320 m^2/g) was from Bischoff Chromatography GmbH (Leonberg, Germany) and was modified with mercaptopropyl groups following a literature procedure.[23] Aqueous ammonia solution (25%), disodium hydrogen phosphate, sodium dihydrogen phosphate, orthophosphoric acid, sodium hydroxide, and methyl isobutyl ketone (MIBK) were purchased from Panreac Química (Barcelona, Spain). Ultrapure water used for mobile-phase preparation was obtained from a MilliQ Academic A10 system. All the buffered mobile phases were filtered under reduced pressure through a 0.45-μm membrane filter.

Instruments. The CPC runs were performed on a high-performance CPC apparatus model LLB-M (EverSeiko, Tokyo, Japan), equipped with a stacked circular partition disk rotor (2136 channels, 220 mL of internal volume). The latter was connected to a Hewlett-Packard 1100 chromatography system. A Rheodyne injector valve with a 2.4-mL loop was employed for manual sample loading. Semipreparative HPLC separation and enantiomeric excess determination were performed on a Merck-Hitachi HPLC system, consisting of a semipreparative pumping system series L-7150, an autosampler series L-7250 equipped with a 100-μL sample loop, a diode array detector series L-7455, and an interface series D-7000. Data processing was performed with D-HSM 7000 software. Determination of the enantiomeric excess of dichlorprop was performed with a Chiral AX-QN1 CSP (125 × 4 mm i.d., CS loading 190 μmol/g) from Bischoff Chromatography.

Synthesis of the $(DHQD)_2PHAL$-Type CSP and CSPA (Figure 2). 1-Chloro-4-(9-*O*-dihydroquinidinyl)phthalazine (1). Dihydroquinidine (10.80 g, 33.0 mmol) was dissolved in dry THF (200 mL), and the solution was cooled in an ice−water bath. Butyllithium (2 M solution in pentane, 17 mL, 33.0 mmol) was added via syringe. Solid 1,4-dichlorophthalazine (8.00 g, 39.6 mmol) was added in a single portion, and the mixture was stirred at ambient temperature. After 5 h, TLC analysis (mobile phase: $CHCl_3$/MeOH, 10:1) showed complete consumption of dihydroquinidine. The solvent was evaporated under reduced pressure at 40°C. The solid residue was dissolved in $CHCl_3$ (200 mL) and washed with water (3 × 200 mL). The organic phase was dried ($MgSO_4$) and concentrated to a volume of 100 mL under reduced pressure. The concentrated solution was purified by flash chromatography on silica gel (300 g), eluting with $CHCl_3$/MeOH, 10:1, to give 12.11 g (24.8 mmol, 75%) of a white solid: mp 194−196 °C; IR (KBr) 2932, 1620, 1538, 1387 cm^{-1}; MS (ESI) 489.3 $[M + H]^+$, 977.5 $[2M + H]^+$, 1467.8 $[3M + H]^+$; ^1H NMR ($CDCl_3$) δ 8.67 (d, 1H), 8.38 (m, 1H), 8.18 (m, 1H), 7.99 (m, 3H), 7.62 (d, 1H), 7.47 (d, 1H), 7.34 (dd, 1H), 7.28 (d, 1H), 3.98 (s, 3H), 3.52 (m, 1H), 2.91 (m, 1H), 2.84 (m, 2H), 2.74 (m, 1H), 2.04 (m, 1H), 1.79 (m, 1H), 1.70−1.42 (overlapped m, 6H), and 0.92 (t, 3H); optical rotation $[\alpha]_{589}$ −184.9°, $[\alpha]_{436}$ −533.7°, $[\alpha]_{546}$ -232.9°, (c 1.0, $CHCl_3$).

1-(9-*O*-Quinidinyl)-4-(9-*O*-dihydroquinidinyl)phthalazine (2). Quinidine (10.41 g, 32.2 mmol) was dissolved in dry toluuene (300 mL). From this solution, a volume of ∼80 mL was distilled to remove traces of water. The remaining solution was added to added to a suspension of **1** (12.11 g, 24.8 mmol) in toluene (80 mL). After addition of finely powdered K_2CO_3 (4.80 g, 35.5 mmol) and KOH (2.40

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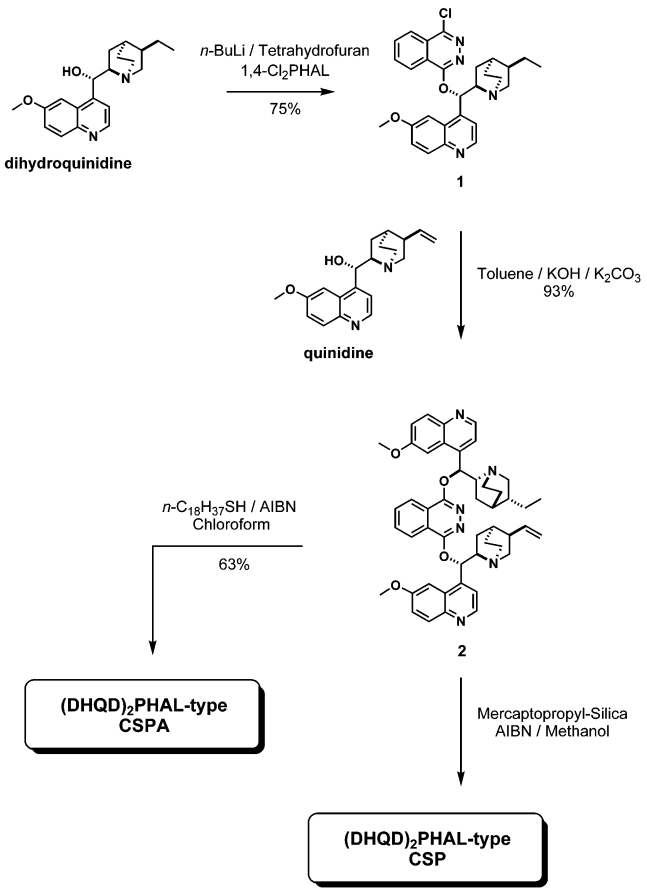

Figure 2. Synthetic scheme for the preparation of the $(DHQD)_2PHAL$-type CSP and CSPA.

g, 43.6 mmol), the mixture was refluxed with stirring for 10 h. TLC analysis of the resulting mixture ($CHCl_3$/MeOH 10:1) showed complete consumption of the starting material. The reaction mixture was allowed to cool, diluted with water (300 mL), and extracted with ethyl acetate (300 mL). The organic phase was washed with brine (2 × 250 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a yellowish oil. Purification by flash chromatography (280 g of silica, gradient elution with $CHCl_3$/MeOH 100/0−90/10) gave 17.90 g (23.0 mmol, 93%) of a white foam: mp 119−122 °C; IR (KBr) 2934, 1621, 1551, 1509 cm^{-1}; MS (ESI) 777.8 $[M + H]^+$, 1554.1 $[2M + H]^+$; ^1H NMR ($CDCl_3$) δ 8.65 (m, 2H), 8.34 (m, 2H), 8.01 (m, 2H), 7.94 (m, 2H), 7.58 (m, 2H), 7.43 (m, 2H), 7.37 (m, 2H), 7.05 (d, 1H), 6.99 (d, 1H), 5.95 (m, 1H), 4.98 (m, 2H), 3.93 (s, 6H), 3.41 (m, 2H), 2.94 (m, 1H), 2.85−2.61 (m, 5H), 2.24 (m, 1H), 2.09 (m, 1H), 1.96 (m, 1H), 1.82 (s, 1H), 1.74−1.36 (m, 12H), and 0.78 (m, 3H); optical rotation $[\alpha]_{589} = -170.1°$; $[\alpha]_{436} = -477.1°$; $[\alpha]_{546} = -214.3°$, (c 1.0, $CHCl_3$).

Immobilization of the Chiral Selector onto Mercaptopropyl-Silica ($(DHQD)_2PHAL$-Type CSP). A 3.0-g amount of mercaptopropyl-modified silica gel[23] was suspended in methanol (40 mL). Precursor **2** (256.0 mg, 330 μmol) and azoisobutyronitrile (5.0 mg, 30 mmol) were added. The mixture was refluxed with gentle mechanical stirring for 5 h. The modified silica gel was isolated by filtration through a sintered glass funnel (porosity 4). The silica gel was washed with $CHCl_3$ (50 mL), $CHCl_3$/MeOH 1:1 (2 × 50 mL), and hot MeOH (4 × 50 mL) and dried in high vacuum at 60 °C. Elemental analysis gave 8.59% C, 1.54% H, 0.69% N, and 2.64% S, corresponding to a CS loading of 82 μmol/g, 0.26 μmol/m^2 (calculated based on N content).

1-(9-*O*-Dihydroquinidinyl)-4-(11-octadecylthia-9-*O*-dihydroquinidinyl)phthalazine ($(DHQD)_2PHAL$-Type CSPA). Precursor **2** (13.60 g, 17.5 mmol) was dissolved in dry chloroform (125 mL). Octadecylmercaptan (25.10 g, 87.5 mmol) and azoisobutyronitrile (150 mg, 0.9 mmol) were added. The mixture was refluxed with stirring for 8 h. The reaction mixture was loaded onto a flash chromatography column (200 g silica) preconditioned with chloroform. After elution of excess octadecylmercaptan with chloroform, the product was isolated by elution with chloroform/methanol, 20:1. Yield, 11.7 g (11.0 mmol, 63%) of a white foam: mp 59−62 °C; IR (KBr) 2928, 2853, 1621, 1592, 1552, 1508 cm^{-1}; MS (ESI) 532.6 $[M + 2H]^{2+}$, 1063.8 $[M + H]^+$, 2128.0 $[2M + H]^+$; ^1H NMR ($CDCl_3$) δ 8.65 (m, 2H), 8.35 (m, 2H), 7.57 (m, 4H), 7.53 (m, 2H), 7.43 (m, 2H), 7.38 (m, 2H), 7.02 (m, 2H), 3.91 (s, 6H), 3.42 (m, 2H), 2.86−2.62 (m, 8H), 2.42 (m, 4H), 2.08−1.92 (m, 2H), 1.78−1.18 (m, 46H), 0.88 (m, 3H), and 0.77 (m, 3H); optical rotation $[\alpha]_{589}$ −147.1°; $[\alpha]_{436}$ −403.9°; $[\alpha]_{546}$ −184.6° (*c* 1.0, $CHCl_3$).

Screening by Liquid−Liquid Extraction. Stock Solutions. The following stock solutions and buffers were prepared for liquid−liquid extraction experiments: 100 mM CSPA (1.063 g of CSPA in 10 mL of chloroform); 100 mM dichlorprop (235 mg of racemic dichlorprop in 10 mL of methanol); 100 mM sodium phosphate and 100 mM ammonium acetate buffers, pH 6.8, 7.0, and 8.0. Prior to use in liquid−liquid extraction experiments, these buffers were equilibrated with MIBK and MTBE, respectively, by shaking in a separation funnel.

Liquid−Liquid Extraction Experiments. A set of 24 glass centrifuge tubes was charged with 200 μL of CSPA and 200 μL of dichlorprop stock solution and dried under a stream of nitrogen. The residues were reconstituted in 2 mL of the respective organic solvent (MIBK or MTBE) and mixed with 2 mL of the respective aqueous buffers. The tubes were sealed with Teflon-lined screw caps and allowed to equilibrate for 6 h at 25 °C with shaking. After phase separation, 250-μL aliquots were withdrawn from the aqueous (lower) phase, transferred into HPLC vials, and diluted with methanol (1.25 mL). The samples were analyzed on a Chiral AX QN-1 CSP (125 × 4 mm i.d.) using methanol/0.1 M ammonium acetate (80:20), pH_a 6.0 as mobile phase. A flow rate of 1 mL/min, a detection wavelength of 230 nm, and a column temperature of 25 °C were used. The retention factor of the first eluted *R*-enantiomer was 5.47 and that of the more retained *S*-enantiomer was 6.61. The dichlorprop concentrations in the individual phases were calculated by comparison with calibration standards. The resultant data were used to calculate CSPA-mediated distribution ratios for the individual enantiomers (D_R; D_S) and the corresponding enantioselective distribution factor α_{ex} (D_S/D_R). The non-CSPA-mediated distribution ratio (D_0) of dichlorprop was performed in an analogous fashion, omitting the CSPA in the organic phase.

CPC Experimental Conditions. Stationary phases for CPC experiments were prepared by dissolving 4.26 g (4.0 mmol) of CSPA in 400 mL of MTBE or MIBK, respectively. The corresponding stationary phase (in all cases the one with lower density) was transferred into the CPC rotor compartment by descending

mode pumping, replacing the previousely charged aqueous mobile phase. As soon as the stationary phase appeared at the outlet, the rotor valve was closed and the rotation (1100−1200 rpm) was started. After 30 min of centrifugation, the respective aqueous mobile phase was pumped into the rotor compartment in descending mode. After initially occurring displacement of stationary phase, clear mobile phase appeared at the outlet. Stabilization of the back pressure indicated that the two-phase system in the rotor had reached a steady-state equilibrium. The volume of stationary phase retained in the equilibrated CPC system was calculated as the difference of the volumes originally loaded and the fraction displaced on introduction of the mobile phase. The amount of racemic dichlorprop to be injected was calculated from the total CSPA amount available in the equilibrated CPC system. Racemic dichlorprop samples corresponding to 0.25, 0.5, 1, and 2 times the molar amount of the total CSPA amount were dissolved in the corresponding organic solvent (2.4 mL) and injected. For all experiments, the flow rate of the mobile phase was set at 3 mL/min. The eluate was collected in 3-mL fractions until the return of the online UV signal indicated complete dichlorprop elution. Due to signal saturation, well-resolved elution profiles could not be obtained with the employed analytical UV detector. Therefore, the corresponding CPC elution profiles were constructed by analyzing the individual fractions by HPLC and plotting the corresponding concentrations of *R*- and *S*-enantiomers versus time. For this purpose, a 500-μL aliquot of each fraction was withdrawn, diluted with 500 μL of methanol, and analyzed by the above-described chromatographic procedure.

Recovery of Dichlorprop Enantiomers and CSPA. The individual dichlorprop enantiomers were recovered from the fractions of the separation performed in MTBE/phosphate buffer at pH 8.0 with equimolar carrier/analyte concentrations as follows. The fractions containing the individual enantiomers were pooled, acidified with concentrated hydrochloric acid, and extracted with chloroform (3 × 100 mL). The combined organic phases were dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. Gravimetric quantification gave recoveries of 165 (90%) and 174 mg (95%) for the *R*-enantiomer and *S*-enantiomer, respectively. For recovery of the CSPA, water was pumped in ascending mode through the system without any rotation. The resulting effluent was saturated with sodium chloride to break the emulsion. Separation of the organic phase, drying over sodium sulfate, and evaporation of the solvents allowed >90% recovery of the CSPA.

HPLC Semipreparative Enantioseparation of Dichlorprop. Semipreparative separation of 4.9, 9.7, and 19.5 mg of racemic dichlorprop was carried out on a 150 × 4 mm i.d. column packed with 1.10 g of $(DHQD)_2PHAL$-type CSP with a loading level of 82 μmol of CS/g. The mobile phase consisted of methanol/acetic acid/ammonium acetate (92:2:0.5, v/v/w). A flow rate of 1 mL/min and a column temperature of 25 °C (controlled with a Haake C40 water thermostat) were used. Peak detection was performed at 300 nm. Samples were dissolved in methanol at a concentration of 100 mg/mL. The enantiomeric excess of each fraction collected was determined as described above.

RESULTS AND DISCUSSION

HPLC versus CPC: Selection of the Model System. Dichlorprop represents one of the most frequently employed chiral herbicidal agents in crop protection, with an estimated annual use of more than 23 000−30 000 tons in the United States.[24] Dichlorprop exhibits pronounced enantioselective biological activity with herbicidal properties being restricted to the *R*-enantiomer, whereas the *S*-enantiomer shows even some anti-auxin effects.[25] The intense and worldwide application of racemic dichlorprop contributes considerable to global pollution, the level of which could be cut down by half using the active enantiomer only. Therefore, the development of efficient enantiomer separation methodologies for this target compound can be considered a highly relevant challenge.

Our efforts to identify enantioselective receptors for environmentally relevant chiral compounds led to the discovery that bis-1,4-(dihydroquinidinyl)phthalazine, a cinchona ligand widely used for asymmetric synthesis protocols,[26] also possesses excellent chiral recognition properties for dichlorprop (Figure 1). Thus, a silica-supported CSP, derived from $(DHQD)_2PHAL$, produced an α_{HPLC} of 15.3 for dichlorprop under optimized chromatographic conditions. This unprecedented enantioselectivity and the ready accessibility of $(DHQD)_2PHAL$ recommend this ligand as a promising CS candidate for the development of bonded CSPs for preparative chromatographic enantiomer separation of dichlorprop. Unfortunately, our attempts to generate high-capacity $(DHQD)_2PHAL$-type CSPs resulted in materials with disappointingly low CS coverages, the best results being in the range of <150 μmol/g. Evidently, the high molecular weight (~760) and the spherical molecular shape of $(DHQD)_2PHAL$ prohibit more densely grafted surfaces due to steric congestion.

Faced with this limitation, we decided to explore the utility of $(DHQD)_2PHAL$ for support-free CPC enantiomer separation, avoiding surface immobilization and allowing for more flexibility in terms of CS loading. Considering environmental issues, we projected a CPC protocol operating with a CSPA-charged organic stationary phase and a purely aqueous mobile phase. Successful realization of this concept called for a $(DHQD)_2PHAL$ species showing excellent solubility in the organic stationary phase while being practically insoluble in the aqueous mobile phase. This crucial requirement was addressed by enhancing the intrinsic hydrophobicity of $(DHQD)_2PHAL$ by covalent attachment of a lipophilizing moiety (Figure 1). To preserve the functional integrity of $(DHQD)_2PHAL$, an octadecylthia group was attached at the C_{11} position of the common precursor, reproducing the molecular microenvironment of the silica-supported CS. The corresponding synthetic aspects are discussed in detail in the next section.

Synthesis. The synthetic routes employed for the preparation of the silica-supported $(DHQD)_2PHAL$-type CSP and the corresponding CSPA from a common precursor are outlined in Figure 2.

Using a modified literature procedure,[22] monochlorophthalazine **1** was prepared in 75% yield from DHQD by deprotonation with butyllithium and subsequent alkylation of the resultant alkoxide with 1,4-dichlorophthalazine. Condensation of **1** with QD under basic conditions provided the common precursor **2** in 93% yield,[22] comprising a single vinyl group as a handle for further functionalization. Reaction of **2** with mercaptopropyl-modified silica gel[23] in the presence of AIBN as a free radical initiator gave the

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(25) Loos, M. A. In *Phenoxyalkanoic acids*; Kearny, P. C., Kaufmann, D. D., Eds.; Marcel Dekker: New York, 1975; Vol. 1, pp 1−101.
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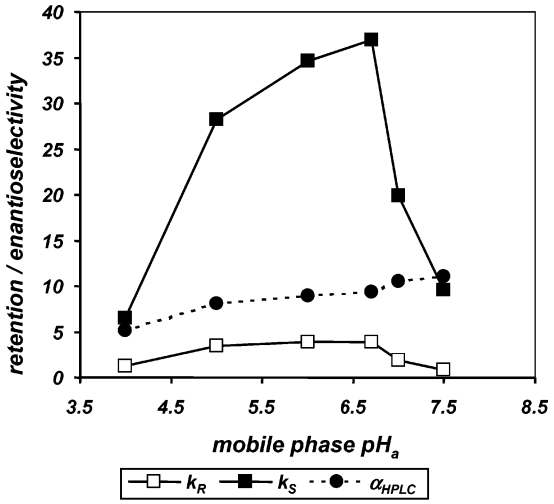

Figure 3. Mobile-phase−pH dependency of the retention factors (k_S, k_R) and the enantioselectivity factor (α_{HPLC}) of dichlorprop on the $(DHQD)_2PHAL$-type CSP. Conditions: column 150 × 4 mm i.d.; mobile phase methanol/20 mM sodium phosphate (80:20, v/v), pH_a adjusted with aqueous orthophosphoric acid (50%) and 6 M sodium hydroxide solutions; flow rate 1 mL/min; UV detection 254 nm; column temperature 25 °C.

corresponding CSP with a CS coverage of 82 μmol/g. For the preparation of the $(DHQD)_2PHAL$-type CSPA, precursor **2** was reacted with an excess of octadecylmercaptan in the presence of AIBN, affording the corresponding highly lipophilic thioether in 61% yield. This compound showed excellent solubility in a wide range of organic solvents (>300 mg/mL for MTBE, MIBK, and $CHCl_3$) but was essentially insoluble in water over a pH range of 5.0−9.0.

Development of the CPC Enantiomer Separation Protocol. Influence of pH on Enantioselectivity/Binding Affinity. To facilitate the development of an efficient CPC enantiomer separation protocol, the impact of operational parameters on enantioselective binding of dichlorprop to $(DHQD)_2PHAL$ was explored. Considering the acid−base character of the receptor−analyte system under investigation, it was of particular interest to establish to what extent enantioselectivity or binding affinity could be controlled by pH variations of the mobile phase. Therefore, the retention of the dichlorprop enantiomers on the silica-supported $(DHQD)_2PHAL$-type CSP was studied in a pH range of 4.0−7.6 with a hydroorganic mobile phase consisting of methanol/20 mM sodium phosphate buffer (80:20, v/v). The corresponding data are depicted in Figure 3.

On increasing the mobile-phase pH, the retention factor of the more strongly bound *S*-enantiomer, k_S, was significantly enhanced, reaching a distinct maximum at pH 6.7. Changing the mobile-phase pH from 4.0 to 6.7 increased k_S from 6.5 to 37.0, while a further increase to pH 7.5 induced a dramatic loss in retention to $k_S = 9.5$. An analogous tendency, albeit far less pronounced, was observed for the less strongly bound *R*-enantiomer. For enantioselectivity, however, a different pH profile was observed. The enantioseparation factor (α_{HPLC}) improved more or less linearly with increasing mobile-phase pH through the entire range studied, showing an enhancement from $\alpha = 5.1$ at pH 4.0 to $\alpha = 11.1$ at pH 7.5.

Although the underlying chiral recognition mechanism is still under investigation, two preliminary conclusions concerning the enantioselective $(DHQD)_2PHAL$−dichlorprop association mechanism can be drawn. The observed pH−retention profile, reflecting most efficient analyte binding at an intermediate pH range, clearly indicates that dichlorprop−$(DHQD)_2PHAL$ complex formation is primarily driven by electrostatic interactions. The contribution of these electrostatic interactions to complex stabilization, however, appears to be largely nonenantioselective in nature, as their partial disruption at the high pH values improves rather than impairs enantioselectivity.

The fact that pH variation affects primarily binding affinity but to a much lesser extent enantioselectivity has important practical implication for selection of the CPC mobile-phase conditions. From the pH−retention profile in Figure 3 it can be concluded that adjusting mobile-phase pH to values of >7 offers a convenient means to tune $(DHQD)_2PHAL$−dichlorprop binding affinity without compromising overall enantioselectivity. Consequently, aqueous phosphate and acetate buffers covering a pH range from 7.0 to 8.0 were selected as mobile phases for further optimization studies.

Liquid−Liquid Extraction Screening. The prudent choice of appropriate mobile/stationary-phase systems is the most crucial task in the development of CPC separation protocols.[27] To guarantee adequate performance in CPC applications, a solvent combination has to fulfill several crucial requirements. Considering the dynamic nature of liquid−liquid partition chromatography, the phase separation kinetics of the stationary/mobile-phase system must be sufficiently fast. In CPC applications, slow phase separation may give rise to the formation of emulsions, leading to an instable separation process or complete displacement of the stationary phase.[27] For enantiomer separation, the solvent system must be fully compatible with the chiral recognition mechanism of the chosen CSPA. The stationary phase must show excellent dissolving capacity for the CSPA and the corresponding CSPA−analyte complexes to ensure high preparative capacity and must completely retain the CSPA under chromatographic operation conditions. Leaching effects would inevitably lead to a continuous loss of CSPA from the stationary phase and, consequently, to system instability and severe contamination of the product fractions. Concerning the analytes, the solvent combination must show a well-balanced partition behavior, with optimal stationary/mobile-phase distribution ratios in the range of 0.2−3.0.[11] Lower distribution ratios correspond to a preferential partition of the analytes into the mobile phase and, thus, poor interaction with the stereodiscriminating CSPA. On the contrary, exceedingly high distribution ratios indicate very strong CSPA−analyte interactions, resulting in loss of peak resolution and impracticably large elution volumes and long run times.

Guided by these criteria, we explored the performance characteristics of different solvent combinations for enantioselective partitioning of dichlorprop in the presence of the $(DHQD)_2$-PHAL-type CSPA by liquid−liquid extraction experiments. To ensure operational simplicity, and to comply with environmental and safety issues, we restricted our choice of organic mobile phases to inexpensive, low-toxicity single solvents. For these reasons, MIBK and MTBE were selected as stationary-phase solvents. These solvents dissolved up to 300 mg/mL CSPA, dichlorprop, and the corresponding diastereomeric complexes at

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Table 1. Influence of Buffer Salts, pH, and Organic Solvents on the Passive (Nonenantioselective) and CSPA-Mediated (Enantioselective) Liquid−Liquid Partition Behavior of Dichlorprop[a]

aqueous buffer	pH	organic solvent	ee_{org}	D_0	D_S	D_R	α_{ex}
phosphate	6.8	MIBK	7.64	0.31	16.7	4.25	3.93
phosphate	7.0	MIBK	15.61	0.13	7.31	1.79	4.08
phosphate	8.0	MIBK	39.82	0.03	1.58	0.36	4.42
acetate	6.8	MIBK	7.55	1.04	24.57	4.75	5.17
acetate	7.0	MIBK	7.22	1.07	15.34	4.33	3.54
acetate	8.0	MIBK	46.02	0.63	1.22	0.26	4.79
phosphate	6.8	MTBE	12.94	0.21	15.02	2.61	5.76
phosphate	7.0	MTBE	29.50	0.28	5.88	0.87	6.76
phosphate	8.0	MTBE	44.65	0.1	1.91	0.33	5.69
acetate	6.8	MTBE	12.92	1.32	18.25	2.72	6.72
acetate	7.0	MTBE	12.19	1.36	15.51	2.78	5.58
acetate	8.0	MTBE	51.61	0.84	2.08	0.27	7.56

[a] A 2-mL aliquot of 100 mM aqueous buffer and 2 mL of 10 mM CSPA−racemic dichlroporp were equilibrated at 25 °C. Phosphate, sodium phosphate; acetate, ammonium acetate; MIBK, methyl isobutyl ketone; MTBE, methyl *tert*-butyl ether; ee_{org}, enantiomeric excess (%) of (*S*)-dichlorprop in the organic phase after extraction; D_0, organic/aqueous distribution ratio of dichlorporp in absence of the CSPA; D_S, D_R, organic/aqueous distribution ratios of (*S*)- and (*R*)-dichlorprop in the presence of 10 mM CSPA; α_{ex}, enantioselectivity of extraction (D_S/D_R).

ambient temperature. For reasons discussed in the preceding section, aqueous sodium phosphate and ammonium acetate buffers, 100 mM, at pH 6.8, 7.0, and 8.0 were tested as mobile phases.

Concerning phase separation kinetics, different behaviors were observed depending on the nature of the buffer salts in the aqueous phase. In the presence of phosphate buffers, all systems showed relatively fast phase separation, being typically complete in less than 30 s. With acetate buffers, phase separation was significantly slower (∼1 min), with a tendency to form short-lived emulsions.

Next we explored passive (in absence of CSPA) and CSPA-mediated liquid−liquid partition of dichlorprop for all possible organic solvent and buffer combinations. For these experiments, equal volumes of organic solvents and aqueous buffers, 10 mM in $(DHQD)_2$PHAL-type CSPA and racemic dichlorprop, respectively, were equilibrated at 25 °C. The efficiency of the enantioselective analyte transfer to the organic phase was determined by quantifying the relative amount and the enantiomeric ratio of dichlorprop remaining in the aqueous phase. From these data, the organic/aqueous distribution ratios for the individual enantiomers (D_R, D_S) and the corresponding enantioselectivity (D_S/D_R) were calculated. In addition, control experiments in the absence of the CSPA were performed to establish the nonselective distribution ratio (D_0) of dichlorprop. The corresponding results are summarized in Table 1.

Inspection of the D_0 values listed in Table 1 reveals that, even in absence of the CSPA, significant amounts of dichlorprop could be extracted into MTBE and MIBK at pH 6.8−7.0. Considering the fact that in this pH range dichlorprop ($pK_a = 2.93$) exists preferentially as a deprotonated and well water-solvated species, this finding is rather unexpected. However, under the experimental conditions employed, both MTBE and MIBK are saturated with the aqueous phase and may contain up to 1.5 and 1.9% (w/w) water, respectively. The presence of water molecules in the organic solvents seems to enhance their intrinsic polarity to an extent that allows solvation of the dichlorprop salts and thus their transfer into the organic phase. The extent of non-CSPA-mediated partitioning into the organic phase was influenced by the nature of the buffer salts and the pH of the aqueous phase. Evidently, the presence of ammonium acetate, as compared to sodium phosphate, greatly facilitated the transfer of dichlorprop into the organic phase. Thus, at pH 6.8, with MTBE a $D_0 = 1.32$ was observed with ammonium acetate, whereas with sodium phosphate extraction into the organic phase was less pronounced ($D_0 = 0.21$). Preferential hydrogen-bonding interactions of the ammonium cation with the acceptor-type organic solvents (and dissolved water) may play a role in this partition phenomenon. At pH 8.0, partition of dichlorprop into the organic phases was largely suppressed for both buffer systems. This may originate from a shift of the dichlorprop dissociation equilibrium in favor of the deprotonated species. The nature of the organic solvent had only a minor influence on the partition behavior, indicating that these media exhibit rather similar solvation capacities for dichlorprop.

Performing the extraction experiments in the presence of the $(DHQD)_2$PHAL-type CSPA induced dramatic shifts in the partition equilibrium of dichlorprop in favor of the organic phase. Generally, the addition of CSPA to the organic phase enhanced the dichlorprop extraction yields by a factor of up to >40. In all cases, (*S*)-dichlorprop was selectively extracted into the CSPA-comprising organic phase, providing impressive enantiomer enrichments up to ee > 50%. The observed preference in enantioselective binding is consistent with the retention characteristics of the bonded $(DHQD)_2$PHAL-type CSP, providing evidence that the extraction and chromatographic processes are governed by closely related chiral recognition phenomena.

The efficiency of the CSPA-mediated partition process was primarily sensitive to the pH value and the nature of buffer salts. For all studied solvent/buffer combinations, extractions performed at pH 6.8 produced the highest levels of dichlorprop enrichment in the organic phase. However, already a rather incremental shift in pH to 7.0 led to a significant drop in distribution ratios, with a most dramatic decrease at pH 8.0. This parallels the pH−retention profile observed for the silica-supported CSP and is indicative of the crucial role of ion-pairing interactions to complex stabilization.

In terms of extraction efficiency, MTBE and MIBK showed similar performance characteristics, with MIBK offering somewhat improved yields at lower pH values. This may reflect more efficient ion solvation properties of MIBK due to its higher dipole moment and dielectric constant. Concerning the buffer salts, the trends were similar to those observed in the absence of the CSPA. Ammonium acetate facilitated dichlorprop transfer into the organic phase relative to sodium phosphate. Again, superior solvation capacity of the employed organic solvents for the ammonium cation may account for this behavior.

The levels of extraction enantioselectivity observed in these liquid−liquid partition experiments, expressed as $\alpha_{ex} = D_S/D_R$, were located in a rather narrow range from 3.9 to 7.6. The nature of the organic solvent had some influence on chiral recognition, with MTBE showing generally improved enantioselective partitioning properties relative to MIBK. In contrast to the extraction yields, α_{ex} was found to be relatively insensitive to pH variation of the aqueous buffer phase.

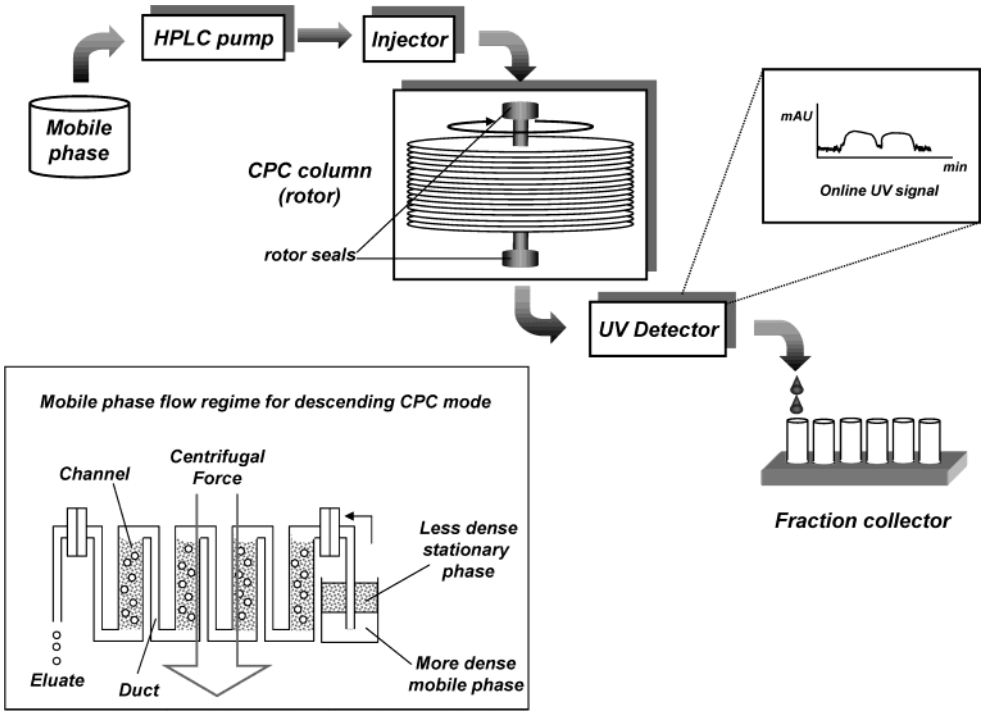

Figure 4. Schematic representation of the chromatographic equipment and (inset) the descending mode mobile-phase flow regime used for the CPC separation of the dichlorprop enantiomers.

As outlined above, solvent systems suitable for CPC enantiomer separation must combine the attributes of high levels of enantioselectivity and well-balanced distribution behavior for the analyte of interest, ideally with stationary/mobile-phase distribution ratios in the range of 0.2−3.0. Inspection of the data summarized in Table 1 indicates that all studied solvent combinations fulfill these criteria in terms of enantioselectivity. Combinations comprising pH 7.0 and 7.5 buffers, however, suffer from exceedingly high distribution ratios and are therefore unsuitable for economical CPC applications. Solvent systems with pH 8 buffers met both requirements in terms of distribution characteristics ($0.26 < D_R, D_S < 2.08$) and enantioselectivity ($\alpha_{ex} = 4.42-7.56$) and were therefore evaluated as mobile phases for CPC separations.

CPC Enantiomer Separation of Dichlorprop. A schematic representation of the CPC apparatus and the operational principle of the employed descendent chromatographic mode are given in Figure 4. The CPC "column" is represented by a rotor assembled from a stack of disks, having an engraved, interconnected system of 2136 partition cells. An individual partition cell consists of a channel and a duct compartment. The channels are the locations where analyte partition through intense mobile/stationary-phase mixing occurs during the chromatographic process, while the ducts provide the transfer of the mobile phase between the channels.

In contrast to HPLC with bonded CSPs, column packing in CPC is generally performed under dynamic conditions. For this purpose, the CPC column is first charged with the stationary phase, followed by the setup of the appropriate operational rotation speed and mobile-phase flow rate. During the initial equilibration stage, the mobile phase displaces a certain fraction of the "gravitationally immobilized" stationary phase until a steady state is reached, depending on the nature of the solvent systems and the operational conditions used. The volume of stationary phase retained under operational conditions in the CPC column defines the total amount of CS available for analyte interactions and thus the preparative capacity of the chromatographic system.

For preparative CPC enantiomer separation of dichlorprop, the solvent systems comprising pH 8.0 buffers and displaying D_R, D_S values in the most economic range of 0.2−2.2, were employed. To ensure good reproducibility of the enantioselective partition efficiencies observed in exploratory liquid−liquid extractions study, all CPC runs were performed with stationary phases comprising 10 mM $(DHQD)_2PHAL$-type CSPA. For the selection of operational parameters, we drew from our experience gained in previous work.[21] A rotational speed of 1100−1200 rpm and a flow rate of 3 mL/min were considered to provide a good compromise among system stability, throughput capacity, and upper pressure limit of the instrument (80 bar).

Under these conditions, the amount of stationary phase (and thus the total amount of CSPA) retained in the CPC column was found to depend on the nature of the organic stationary-phase solvent. With MTBE-comprising solvent systems, stationary-phase retention was 155 mL (70% of the total column volume), while with the MIBK systems, only 130 mL (59% of the total column volume) was retained. These different levels of CSPA loading were considered when the sample amounts to be processed in the preparative CPC runs were calculated.

To identify the most suitable solvent systems for the preparative enantiomer separation, a series of CPC chromatographic experiments was performed with the set of selected solvent systems and increasing amounts of racemic dichlorprop. Comparative runs conducted with the ammonium acetate/MIBK and phosphate/MIBK systems and a sample load corresponding to a molar dichlorprop/CSPA ratio (r) of 0.25 both produced clean baseline separation (data not shown). However, the CPC run performed with acetate buffer as mobile phase showed significantly increased analyte retention (acetate buffer $k_R = 1.27$, $k_S =$

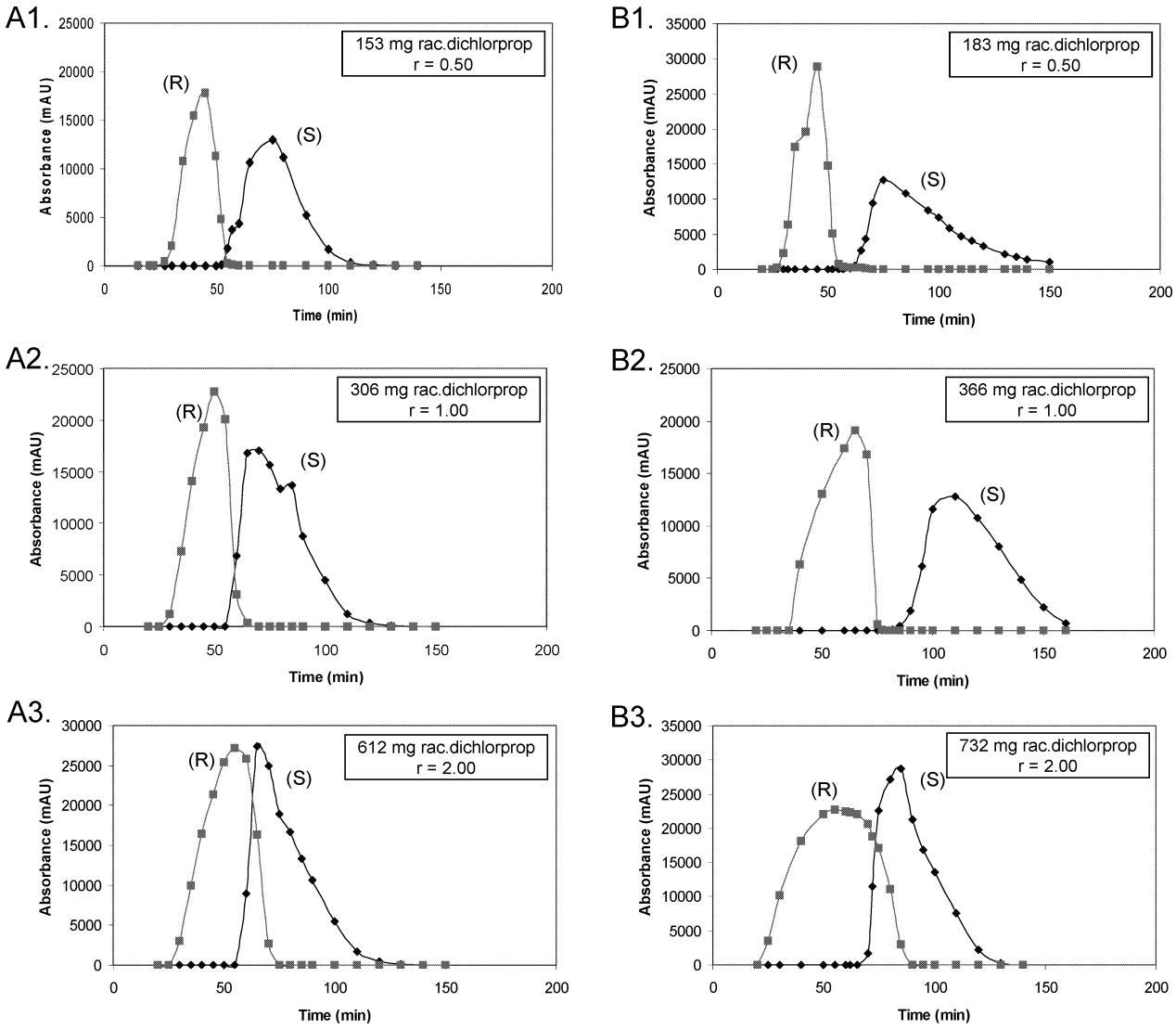

Figure 5. Preparative CPC enantiomer separation of dichlorprop performed with increasing sample loads under different stationary-phase conditions. All experiments were carried out at a flow rate of 3 mL/min with aqueous sodium phosphate buffer (100 mM, pH 8.0) as mobile phase at a rotor speed of 1100 (MTBE) and 1200 rpm (MIBK), and a temperature of 25 °C. Series A: 10 mM $(DHQD)_2$PHAL-type CSPA in MIBK as stationary-phase solvent. Series B: 10 mM $(DHQD)_2$PHAL-type CSPA in MTBE as stationary-phase solvent. The amounts of racemic dichlorprop injected are indicated in the framed boxes. *r* refers to the molar ratio of the loaded amount of racemic dichlorprop and the total amount of CSPA present in the CPC rotor.

6.28; phosphate buffer $k_R = 0.36$, $k_S = 1.27$), indicating less efficient mass transport properties. In context with preparative applications, extensive retention compromises the overall productivity of the process by limiting throughput and increasing solvent consumption. As a consequence, solvent combinations with acetate buffers were excluded from further studies.

With the phosphate-buffered mobile phase, both the MIBK and the MTBE stationary-phase system showed favorably fast elution behavior. Thus, clean baseline enantiomer separation could be achieved with sample loads corresponding to a molar analyte/CS ratio $r = 0.5$ (see Figure 5, A1 and B1), indicating excellent preparative capacities for both systems.

The excellent preparative capacity motivated us to probe the CPC performance under equimolar sample loading conditions ($r = 1.0$, Figure 5, A2 and B2). Much to our delight, the phosphate/MTBE system provided, even under these challenging conditions, complete enantiomer separation. With the phosphate/MIBK combination, minor peak overlap was observed, resulting in a few mixed fractions containing 14% of the injected sample.

Finally, the sample loading was further increased to $r = 2.0$ (Figure 5, A3 and B3). Achieving baseline separation under these loading conditions would realize the economically highly desirable scenario of "total CS exploitation", a situation in which every single CSPA molecule physically effects the separation of a pair of enantiomer molecules. Enantiomer separations were incomplete with both solvent systems under these conditions; however, peak overlap was moderate (38 and 21%, respectively), permitting isolation of major fractions of the injected racemic sample as pure enantiomers.

Enantiomer Separation of Dichlorprop on CSP1. The exceptional preparative performance observed with the $(DHQD)_2$-PHAL-type CSPA under CPC conditions motivated us to carry out a comparative HPLC study, employing the corresponding CSP with the parent cinchona-type CS covalently linked onto the

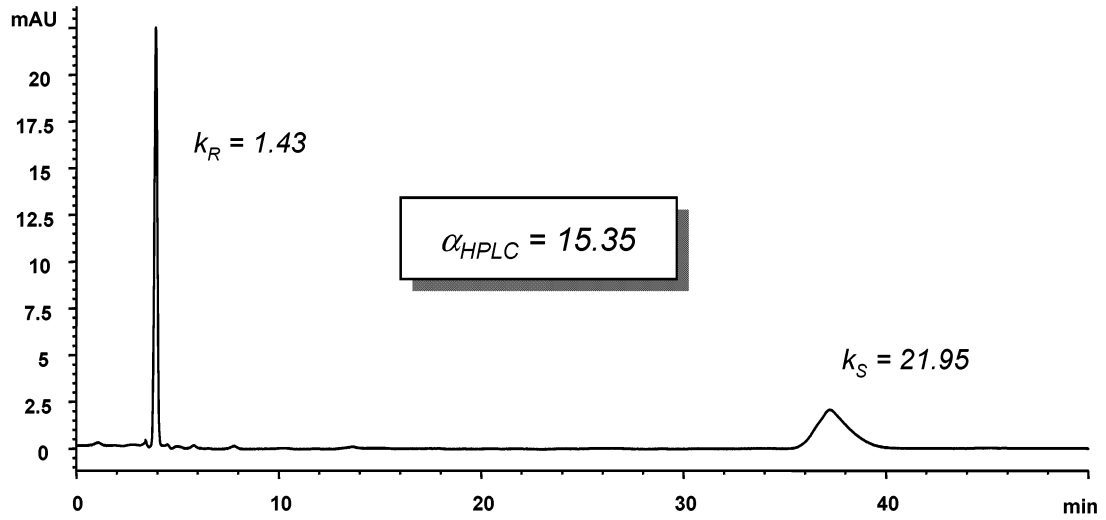

Figure 6. Analytical HPLC enantiomer separation of dichlorprop on the $(DHQD)_2$PHAL-type CSP under optimized mobile-phase conditions: column (150 × 4 mm i.d.); mobile phase methanol/acetic acid/ammonium acetate (92:2:0.5, v/v/w); flow rate 1 mL/min; UV detection 254 nm; column temperature 25°C; injected sample amount 20 μg.

surface of 5-μm spherical silica particles. The $(DHQD)_2$PHAL-type CSP was available as an analytical column (150 × 4 mm i.d.) only, containing 1.10 g of CSP with a CS loading level of 82 μmol/g. The mobile-phase conditions employed in CPC were found to be incompatible with HPLC separation due to the extreme analyte retention (k_R > 10) and prohibitively high back pressure (>150 bar at a flow rate of 1 mL/min) observed. Optimization of the mobile-phase conditions identified a mixture of ammonium acetate and acetic acid in methanol as a favorable alternative. This mobile phase provided the benefits of excellent enantioselectivity (α_{HPLC} > 15; see Figure 6), along with acceptably short retention times and workable back pressure (60 bar).

Employing these polar organic mode conditions, the $(DHQD)_2$-PHAL-type CSP was challenged with increasing amounts of racemic dichlorprop corresponding to *r* values of 0.25, 0.50, and 1.0, respectively. The collected product fractions were analyzed with respect to enantiomer purity. The chromatograms of these preparative runs, and the corresponding quality control runs, are depicted in Figure 7. Inspection of the elution profiles of the preparative runs reveals a particular behavior in terms of peak shapes: While sample overloading induced strong peak distortion for the more retained *S*-enantiomer, the peak quality and sharpness of the first-eluted enantiomer was almost unaffected. Baseline and near-baseline enantiomer separations could easily be achieved for 4.9 mg ($r = 0.25$). and 9.7 mg ($r = 0.50$) racemic dichlorprop.

For the highest sample load tested, i.e., 19.5 mg of racemic dichlorprop corresponding to $r = 1.0$, the peaks merged completely into a single unstructured band, suspending the possibility for UV signal-guided fractionation. However, due to the favorable "peak compression" of the first-eluted *R*-enantiomer, time-controlled peak fractionation still allowed isolation of enantiomerically highly enriched product fractions. The analytical assessment of the enantiomeric excess values of the collected fractions showed ee > 98% for the first-eluted *R*-enantiomer and >96% for the more retained *S*-enantiomer. In the case of the highest sample loading, however, poor peak resolution resulted in a somewhat reduced enantiomer purity for the less retained enantiomer (ee > 92%).

Preparative Capacity of CPC versus HPLC. The application of cogeneric $(DHQD)_2$PHAL-type CS systems for HPLC—as well as CPC-based enantiomer separation—offers the opportunity to assess merits and limitations of these complementary techniques. For this purpose, an overview of the corresponding operational parameters, specific productivities, and mobile-phase consumptions for CPC and HPLC runs performed with a $r = 1.0$ is given in Table 2. The data given for CPC refer to run B2 in Figure 5, and for HPLC, to run C in Figure 7.

The most significant finding emerging from this comparative study is that CSPA-mediated CPC shows a level of *CS utilization* comparable to that of CSP-based HPLC. Evidently, both chromatographic technologies allow exceptionally high levels of loadability, with complete dichlorprop enantiomer separations still achievable with racemic sample amounts equimolar to the incorporated CS ($r = 1.0$).

Significant differences in the performance characteristics of CPC and HPLC, however, become obvious when comparing productivity-related figures. Considering the different molar amounts of $(DHQD)_2$PHAL employed in CPC and HPLC experiments, the productivity data and mobile-phase requirements observed on the analytical column were appropriately normalized with respect to the effective CS concentration, according to well-established linear scale-up rules.[28] Thus, relating the mass of resolved racemate to the molar amount of applied CS and time, HPLC should be capable of separating roughly 7.8 kg of racemic dichlorprop per mol of CS per day, while CSPA-mediated CPC can only separate 2.1 kg per mol of CS per day.

Inspection of the operational parameters listed in Table 2 identifies the mobile-phase flow rate as the major productivity-limiting factor. The rather slow flow rate (3 mL/min) employed in CPC leads to extended run times (160 min) compared to HPLC (40 min) and thus to decreased throughput capacity. The possibility to enhance CPC productivity by increasing flow rate seems to be limited as this may reduce the volume of stationary phase retained within the CPC column and thus compromise preparative enantiomer separation capacity. In addition, higher flow rates may increase the back pressure beyond the instrumental limit (80 bar).

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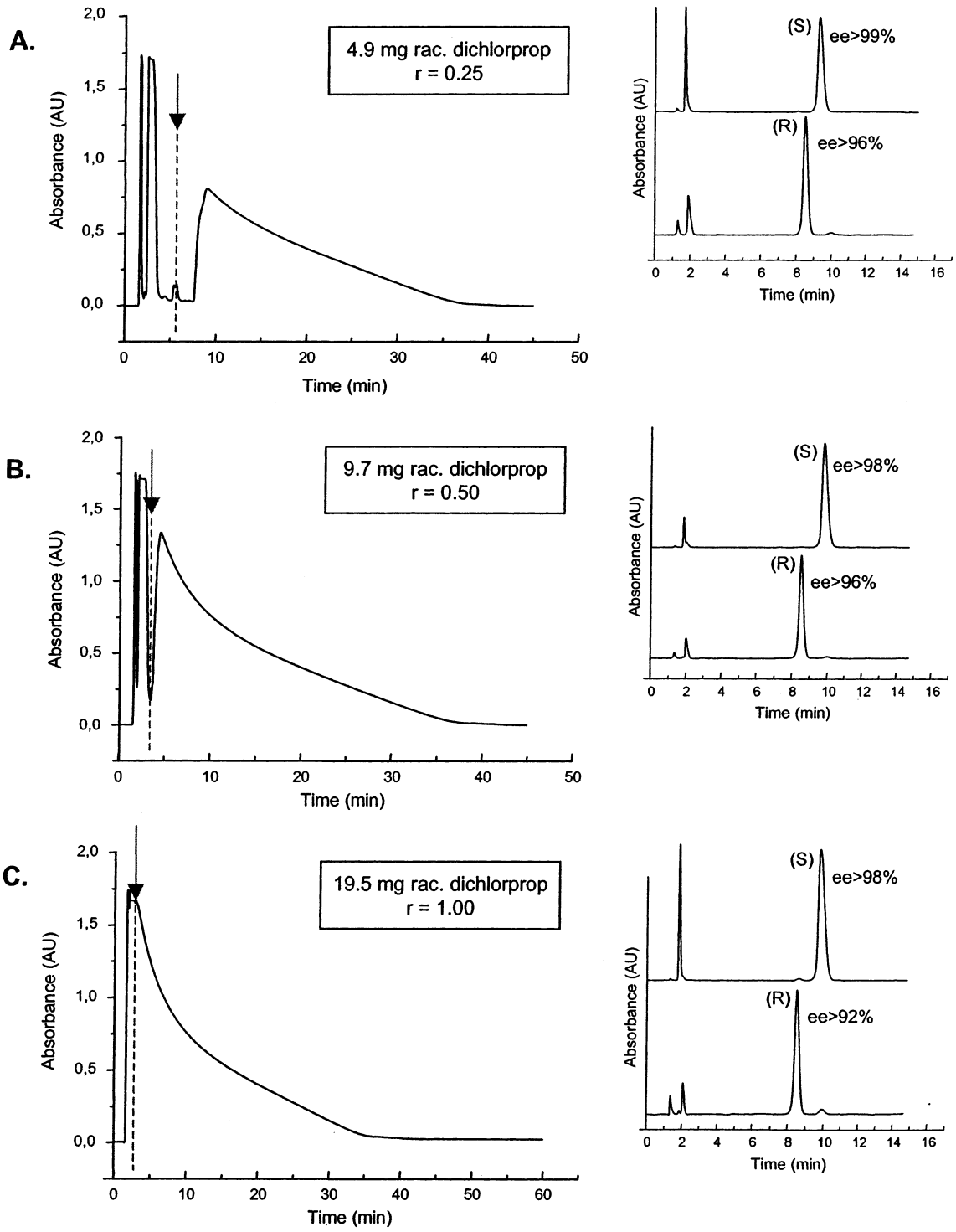

Figure 7. Preparative HPLC enantiomer separation runs performed with the $(DHQD)_2PHAL$-type CSP with increasing sample loads of racemic dichlorprop. The fractionation times are indicated by the broken lines. The chromatograms depicted in the right column give the enantiomeric ratios of the collected fractions, obtained with a different type of CSP (see Experimental Section). The amounts of racemic dichlorprop injected are indicated in the framed boxes. *r* refers to the molar ratio of the loaded amount of racemic dichlorprop and the total amount of immobilized $(DHQD)_2PHAL$-type CS present in the column. For experimental conditions see information given for Figure 6.

With respect to solvent consumption, the developed CPC separation protocol is much more economical than HPLC. The mobile-phase consumption of CPC amounts to 1.30 L/g of resolved racemate, while under HPLC conditions, 2.10 L/g of resolved racemate is required. Apart from its lower solvent consumption, the CPC separation protocol also appears to be more attractive from an environmental viewpoint. In contrast to HPLC, which operates with a mobile phase largely composed of methanol, CPC enantiomer separation can be achieved with an aqueous phosphate buffer mobile phase. Thus, with CPC, the utilization of organic

Table 2. Comparison of the Preparative Performance Characteristics of Developed CPC and HPLC-Based Enantiomer Separation Protocols for Dichlorprop[a]

	CPC	HPLC
stationary phase	10 mM CSPA in MTBE (155 mL)	CSP grafted with 82 μmol of CS g^{-1} (1.10 g)
total amount of CS (mmol)	1.55	0.092
mobile phase	100 mM sodium phosphate, pH 8.0	methanol/acetic acid/ ammonium acetate 92/2/0.5 (v/v/w)
racemate resolved per run (mg/mmol)	366/1.55	19.5/0.083
r	1.0	1.0
ee of isolated *S*/*R*-enantiomer (%)	98/98	98/92
recovery (%)	>95	>90
mobile-phase consumption per run (mL)	480	40
run time (min)	160	40
productivity (kg of racemate d^{-1} mol^{-1} CS)	2.1	7.8[b]
solvent consumption (L g^{-1} racemate)	1.30	2.10[b]

[a] *r*, molar ratio of the racemic dichlorprop loaded and the chiral selector incorporated in the separation system. [b] These figures were calculated for a column comprising an equivalent amount of $(DHQD)_2PHAL$-type CSP, according to well-established linear HPLC scale-up rules.[28]

solvents is reduced to a minimum (i.e., the amount of MTBE used as stationary-phase solvent), allowing for significant savings in chemicals and waste stream management.

We wish to point out that the above figures were calculated under ideal "linear upscaling conditions". In practice, upscaling efforts may be associated with severe limitations. These may include, for example, the need of handling excessively large stationary- and mobile-phase volumes, the limited availability of large-scale CPC instruments, and the intrinsic loss of HPLC efficiency on changing to CSP materials of larger particle size. Thus, the productivity data outlined in Table 2 reflect "best case scenarios", rather than real world situations.

CONCLUSIONS

A support-free CPC enantiomer separation protocol for dichlorprop was developed utilizing a purposefully designed CSPA derived from $(DHQD)_2PHAL$. The selection of solvent systems suitable as stationary/mobile phases for CPC application was guided by enantioselective liquid−liquid extraction experiments in the presence of the $(DHQD)_2PHAL$-type CSPA. A system consisting of 10 mM CSPA in MTBE/sodium phosphate buffer (pH 8.0) was identified to provide the benefits of high enantioselectivity, well-balanced distribution behavior, and satisfactory system stability. Preparative CPC runs performed with this solvent combination showed excellent loadability, producing clean baseline separations with sample loads up to amounts equimolar to the CSPA present in the stationary phase. Comparison with the HPLC performance characteristics of a silica-supported version of $(DHQD)_2PHAL$ revealed that CPC offers a comparably high preparative loadability at significantly reduced solvent consumption. CPC, however, gave lower specific productivity, mainly imposed by instrument-inherent flow rate restrictions. These limitations in productivity may be overcome by (i) enhancing system loadability by increasing the CSPA concentration in the stationary phase and (ii) improving peak capacity through pH zone refining.[11,16]

A serious drawback currently prohibiting the broad use of CPC technology for preparative enantiomer separation is the lack of suitable CSPAs and the technically still immature state of large-scale CPC instrumentation. Efficient CSPAs for preparative CPC application must exhibit high levels of target-specific enantioselectivity, excellent solubility, and complete retention in the stationary-phase media and readily tunable binding affinity to control analyte distribution behavior. Concerning instrumentation, CPC equipment is less readily available and significantly more expensive as compared to HPLC systems. Adaptation of CPC enantiomer separation to industrial-scale application appears to be more challenging than for HPLC, particularly in view of engineering continuously operating process configurations.

Nevertheless, the impressive preparative performance of the model CPC enantiomer separation protocol presented in this paper justifies further research in these directions. The possibility to achieve efficient enantiomer resolution with support-free CSs may help master preparative separations under conditions incompatible with conventional silica-supported CSPs, for example, with salt-rich aqueous mobile phases at extreme pH values. The excellent compatibility of CPC with aqueous mobile phases may also provide unique opportunities for the implementation of low-cost and environmentally friendly enantiomer separation schemes.

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