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Note

Separation of enantiomers using polymeric copper–amine–cellulose columns

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Though always intellectually challenging, with the current high interest in biotechnology, the problem of separating and analyzing enantiomeric mixtures is one of high importance. Liquid chromatography (LC) has been investigated extensively for the convenient and rapid separation of enantiomers. Several LC methods have been applied for the separation of enantiomers, namely, the use of chiral mobile phase additives, the use of chiral stationary phases (Pirkle columns), ligand exchange, charge transfer complex formation and inclusion complex formation with cyclodextrins[1–3]. It has been demonstrated that ligand-exchange chromatography (LEC) is very effective for the separation of optical isomers without prior derivatization[4]. LEC involves the use of a chiral ligand and copper(II) in the stationary phase or mobile phase and the optical isomers are separated primarily by the difference in the stabilities of the copper(II)–chiral ligand–enantiomer complexes. The major problems with the existing LEC columns are, (1) they are difficult to prepare and consequently expensive and (2) the separated analytes are contaminated with Cu^{II} either due to the addition of Cu^{II} to the mobile phase or the leaching of Cu^{II} from the stationary phase. We have addressed these problems in the current work.

We have developed in our laboratory several polymeric Cu^{II}–amine–cellulose complexes, where amine is diamine like 1,3-diaminopropane and cellulose is unmodified or modified cellulose[5]. Cu^{II} on the average is complexed to one glucose unit of cellulose and one amine molecule in these complexes. Cellulose provides the chiral environment necessary for the separation of optical isomers, which in combination with Cu^{II} becomes an excellent choice as column material. These compounds are easy to prepare, inexpensive and can be coated with ease in various amounts to obtain different loadings of Cu^{II} on a variety of substrates like silica and polystyrene. Columns for both analytical- and preparative-scale separations can be thus readily made. Further, as described in the Experimental section, these columns are stable under a variety of conditions exhibiting insignificant leaching of Cu^{II}. Cellulose and its derivatives as well as other polysaccharides like Sephadex have been used as chiral stationary phases for the separation of optical isomers[6,7]. However, these separations were not achieved by LEC and were not very efficient. We have achieved excellent

separation of D- and L-alanines from a racemic mixture using copper^{II}–1,3-diaminopropane–cellulose coated on silica by LEC. The experimental procedure and results are described here.

EXPERIMENTAL

Reagents

The alanines (D, L and the racemic mixture), $CuSO_4 \cdot 5H_2O$ and 1,3-diaminopropane were obtained from Aldrich and were >99% purity. High-purity cellulose of average particle size 10 μm without any binder, suitable for thin-layer chromatography (manufactured by Machery, Nagel & Co., F.R.G., and distributed by Brinkman Instruments, New York, U.S.A.) was used to prepare the Cu^{II}–1,3-diaminopropane–cellulose complexes. Silica gel (70–150 μm) was from Analtech. All the solvents used were HPLC grade and were purchased from Mallinckrodt. Deionized water for the HPLC experiments was distilled twice over permanganate.

Apparatus

The separation of D- and L-alanines were performed on a Perkin-Elmer Series 4 liquid chromatograph with Rheodyne 7125 injector containing a 20-μl loop, a Perkin-Elmer LC 95 UV–visible spectrophotometric detector and Alltech Linear 1200 strip-chart recorder. The leaching of Cu^{II} under the various experimental conditions used for the separation of the racemic alanine was checked using the Perkin-Elmer 6500 inductively coupled plasma–atomic emission spectrometry (ICP–AES) system at the 224.7 nm emission wavelength (detection limit: 0.2 ppm).

Procedure

The Cu^{II}–1,3-diaminopropane–cellulose complex was prepared as previously reported[5]. The proposed structure for this complex is shown in Fig. 1. Silica gel, 15.5 g, was washed three times with deionized water, three times with methanol and finally with deionized water. It was stirred with 50 ml of Cu^{II}–1,3-diaminopropane–cellulose complex containing $8.8 \cdot 10^{-3}$ mol of Cu^{II} for 1 h and allowed to settle. A blue colored

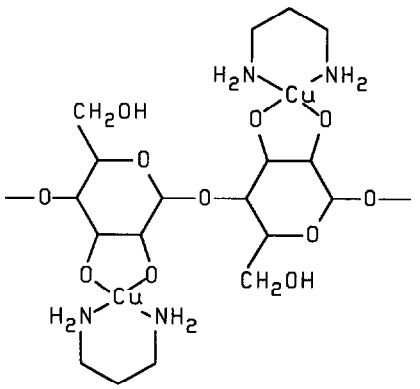

Fig. 1. Proposed structure for copper(II)–1,3-diaminopropane–cellulose.

gel and a clear aqueous layer were obtained, indicating that the silica gel has been coated with the complex. The gel was filtered on a medium frit funnel, washed several times with distilled water and dried in vacuum at room temperature for 12 h. The blue colored silica gel, 1.2 g (Cu^{II} = $5.6 \cdot 10^{-4}$ mol/g of silica), was slurried with methanol and packed into a stainless-steel high-performance LC column of 28 cm × 0.3 cm I.D. at a flow-rate of 1 ml/min at room temperature. No significant amount of Cu^{II} was found to leach from the column with water (pH 3–9), 0.1 *M* NH_3, methanol and acetonitrile as eluents. This was determined by analyzing the eluent for Cu^{II} using ICP–AES. Water solutions were directly analyzed while organic solvents were evaporated and the container washed with an appropriate volume of water for analysis by ICP–AES. Several eluent compositions were tried to obtain the best separation for a solution containing 10^{-3} *M* (89 μg/ml) racemic DL-alanine in water at pH 7

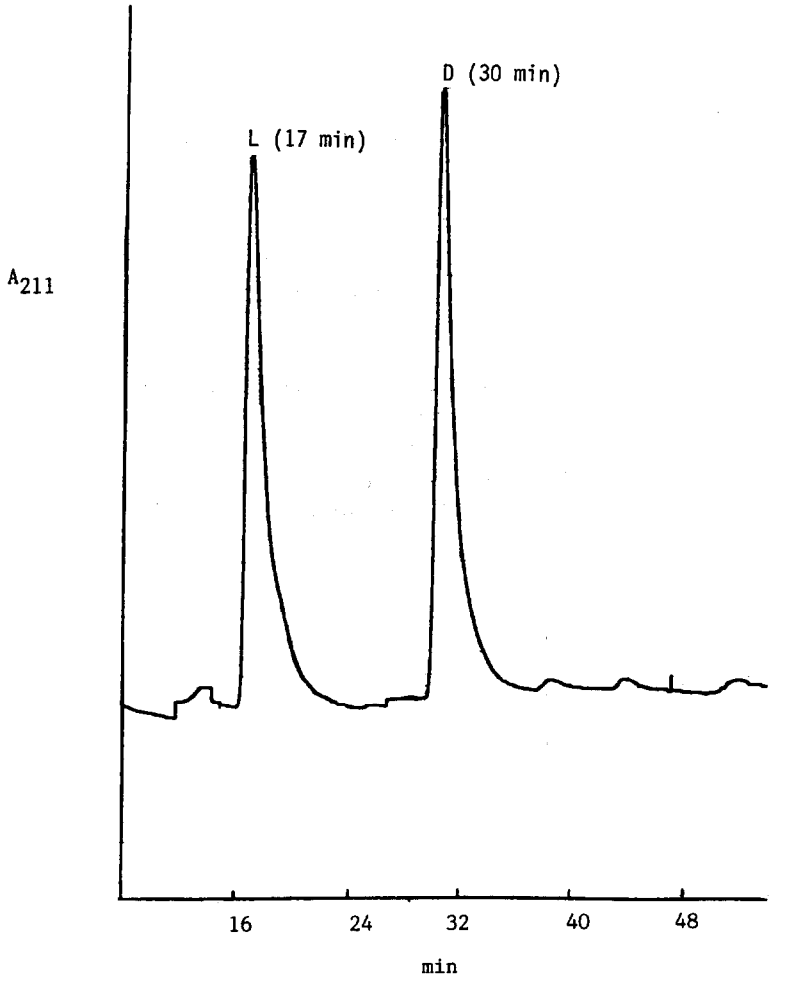

Fig. 2. Separation of DL-alanine using copper(II)–1,3-diaminopropane–cellulose coated on silica.

(phosphate buffer). An eluent composition of methanol–water–acetonitrile (80:10:10) at a flow-rate of 1 ml/min afforded the best separation of the D and L forms. The detection wavelength was 211 nm. The individual components were identified by injecting separately D- and L-alanine of the same concentration as the mixture. The retention time for the non-adsorbing analyte acetone under these conditions was 1.7 min.

RESULTS AND DISCUSSION

Excellent baseline separation of the D- and L-alanines from the racemic mixture was obtained under the experimental conditions as shown in Fig. 2. The retention times are 17 and 30 min for L- and D-alanines, respectively. The corresponding capacity factors using the retention time for the non-adsorbing analyte acetone for L- and D-alanines are 9.0 and 16.7, respectively. These results yield a separation factor α of 1.85. The separation factors for the separation of racemic DL-alanine using some other columns reported in the literature are collected in Table I. As may be seen from this table, the separation factors obtained using the Cu^{II}–1,3-diaminopropane–cellulose coated on silica are comparable or superior to these other columns. The most significant advantage to the column we have developed is the simplicity of its preparation. Application of this column for the separation of several other racemic compounds are currently under investigation.

We may also notice from Fig. 2 that the peaks for L- and D-alanines have about the same width, *i.e.*, the number of theoretical plates calculated from the peak widths using the equation for asymmetric peaks are markedly different (373 for the L isomer and 1162 for the D isomer)[13]. This difference could be rationalized using the Knox equation for reduced plate height h[14]. Stout *et al.*[15] have shown that the B and C terms of the Knox equation which account for the diffusion and mass transfer of the analyte

TABLE I

LITERATURE VALUES FOR SEPARATION FACTOR (α) OF D- AND L-ALANINES

Column material	α	*Ref.*
Polystyrene derivatized with (*R*)-N',N'-dibenzyl 1,2-propanediamine	1.43	8
Silica gel derivatized with (*R*)-N-[11-(triethoxysilyl)undecanoyl]-α-(6,7-dimethyl-1-napthyl)isobutylamine	1.28 (as the N-3,5-dinitrobenzoyl derivatives)	9
Silica gel derivatized with N-ω-(dimethylsiloxy)undecanoyl-L-valine	2.34 (as the 5-dimethylaminonapthalene-1-sulphonyl derivatives)	10
Polystryene derivatized with L-methionine-*l*-sulphoxide	1.19	11
Lichrosorb RP-18 with Cu^{II} and N,N-dimethyl-L-phenylalanine in the eluent	1.75	12
Silica gel derivatized with β-cyclodextrin	1.20	2

depend on its capacity factor, B increasing and C decreasing with the capacity factor. The magnitude of the change in C can be much higher than B if the diffusion of the analyte within the intraparticle region is impeded by steric effects and/or pore size of the solid support. The three-point interaction that is generally believed to be responsible for chiral recognition by ligand exchange indicates that the most likely contributing factor for our columns is steric effect[16]. As a result the h value for the more retained D-alanine is smaller and hence its plate number larger compared to the less retained L-alanine. This aspect is under further investigation.

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