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Journal of Chromatography A, 868 (2000) 189–196

JOURNAL OF CHROMATOGRAPHY A

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# Chiral and electrokinetic separation of amino acids using polypyrrole-coated adsorbents

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Received 3 August 1999; received in revised form 19 October 1999; accepted 19 November 1999

**Abstract**

An optically active and electroconductive polymeric adsorbent has been developed for the use in chromatographic resolution of nonderivatized amino acids. The chiral selectivity of the adsorbent is based upon ligand exchange of coordinated copper(II) complexes of D or L-amino acids and a molecular imprinting technique by modifying the resin surface with polypyrrole coating. Applying a potential difference of ±1.5 V to the chiral and conductive column, racemic amino acids are separated according to their charge characteristics, and simultaneously resolved with respect to their optical isomerisms. A pH-controlled mixture of D,L-lysine and D,L-aspartic acid is resolved displaying enantioselectivity values of 1.19 and 2.08, respectively, and a baseline separation of the two amino acids is accomplished by alternating the polarity of the electric field. The synthesized adsorbent also exhibits size exclusion factor discriminating amino acids with larger side chains. 

*Keywords:* Enantiomer separation; Molecular imprinting; Amino acids

## 1. Introduction

Amino acids and their derivatives are chiral, and with few exceptions only one enantiomer is of interest [1]. A number of fundamentally different processes are available for purifying amino acids with regard to both the type of amino acid and the enantiomeric form. Since a ligand exchange adsorbent for the resolution of chiral amino acids was introduced by Davankov's group [2], various inventions based upon similar molecular recognition principles have been introduced [3,4]. Davankov-type chiral resins utilize the heterobinuclear complex formation of D,L-amino acids by transition metal ions such as Cu(II), and the chiral selector is commonly a Cu(II)-chelated proline molecule for its five membered ring is free of rotation contributing assured steric effects [5]. The differentiation of enantiomers was possible by the ''three-point interaction'' between the stationary phase and the mobile ligand [6,7]. In an effort to enhance the separation efficiency, this type of ligand exchange adsorbents coupled with molecular cavities of stereospecificity to the targeting amino acid enantiomers were recently developed [7,8]. The method involved preparation of a synthetic polymer of desired affinity for a certain molecule (molecular imprinting) [7].

Amino acids are amphoteric compounds. They can be anions, cations, and zwitterions depending upon the solution pH [1]. Electrokinetic separation is one

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0021-9673/00/$ – see front matter 
PII: S0021-9673(99)01246-7

of the promising ways to separate amino acids of different isoelectric points. Use of an electrokinetic version of ion-exchange chromatography system is most beneficial when amino acids of different electrovalencies are to be efficiently separated by controllable ion-exchangeability of the column. Electrokinetic ion-exchange columns, where an electroconductive stationary phase and working electrodes are employed, have been used in heavy metal recovery (the Nalco process) [9] and separation of various ionic compounds [10,11]. The electrosorption/elution can be finely controllable by adjusting the applied electric filed strength and/or applying voltage wave forms to the conductive stationary phases.

By taking advantage of the two methods of separation described above, we have developed a different synthetic route to prepare a molecularly imprinted conductive adsorbent. The preparation method involves a ligand exchange resin with Cu(II) complexes, and an additional coating process with polypyrrole. When in use as an electrochemical liquid chromatography column, this adsorbent allows differential sorption/elution of amino acids based on their electrochemical characteristics as well as stereochemistry.

## 2. Experimental

### 2.1. Synthesis of chiral and conductive adsorbent

An optically active polymer resin was prepared by following the reaction scheme suggested by Davankov and Rogozhin (1974) [5]. Chloromethylated poly(styrene-*co*-divinylbenzene) (Merrifield resin [12]) was purchased from Aldrich (Milwaukee, WI, USA). This polymer was treated with 2.25 mol of L-proline and 0.30 mol NaI (Aldrich) per 1 mol of chloromethylated groups. After washing and drying in vacuum overnight, the chloromethylated polystyrene was treated with 0.1 *M* $CuSO_4 \cdot 5H_2O$ in 0.5 *M* $NH_4OH$, following which 0.1 *M* D-proline solution at pH 7.5 was applied. This D-proline was the print molecule. After a reaction time of 1 h followed by washing and drying, 0.5 g of this sorbent with Cu(II) chelates was deposited in a polymerization bath composed of 0.04 *M* pyrrole and 0.012 *M* *p*-toluenesulfonic acid in methanol at 0°C. Upon introducing aqueous 0.1 *M* $FeCl_3$ to the polymerization bath, the chemical polymerization of pyrrole was carried out for 45 min. Filtering and sieving the polymerization mixture, 200–400 mesh particles were resulted.

### 2.2. Adsorption isotherm

The adsorption isotherms of D- and L-proline on the imprinted ligand exchanger were constructed by batch adsorption experiments. Both amino acid solutions were prepared in 1.5 ml of 5 m*M* sodium acetate and 5 m*M* $NH_4OH$ solution at pH 7.0 at various concentrations, to which 0.05 g of the Cu(II)-loaded adsorbent was added. The slurry was equilibrated for 12 h. After centrifugation the supernatant was analyzed by an HPLC system (VISTA 5560 with CHO-620 $Na^+$-form column, Varian, Sugar Land, TX, USA) with a refractive index detector (RI-4, Varian).

### 2.3. Chromatography system

A porous Vycor glass tubing (I.D. 4.3×95 mm in length, BAS, West Lafayette, IN, USA) of average pore size of 5 nm was packed with 0.95 g of the polypyrrole-coated adsorbent (designated as column 1). As shown in Fig. 1, the Vycor column was positioned concentrically inside a polypropylene tubing where platinum wires were implanted for the electrical contact. This primary column with the working electrodes was connected to two auxiliary columns which were individually packed with 0.45 g of the polypyrrole-coated chiral sorbent (column 2) and 0.32 g of the proline methyl polystyrene free of polypyrrole coating (column 3). Column 1 was connected to a DC power supply (Hewlett-Packard, Palo Alto, CA). Column 2 provided further resolution of racemic amino acids eluting from column 1, and column 3 captured the eluting Cu(II) ions from columns 1 and 2.

The mobile phase was 5 m*M* sodium acetate and 5 m*M* $NH_4OH$ in 1 m*M* EDTA, which provided the background electrolytes for the electric field generated in column 1 as well as the pH buffering. The pH was adjusted with acetic acid to 7.0, where the average molecular charges of lysine and aspartic acid

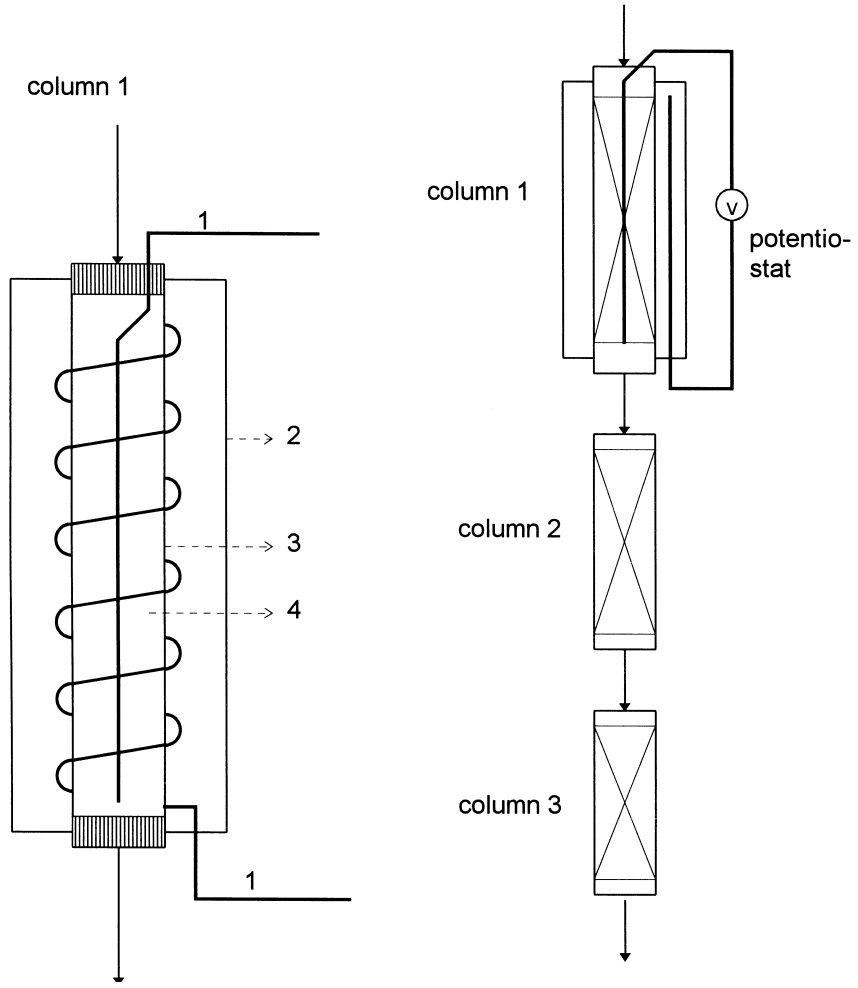


Fig. 1. Electrochemical chromatography system for the chiral separation of nonderivatized amino acids consisting of triple columns in series. Column 1 and 2 are packed with molecularly imprinted polypyrrole/proline methyl poly-styrene composite adsorbent. Column 3 is packed with proline methyl styrene free of polypyrrole coating and Cu(II) [(1) Pt electrode; (2) polypropylene housing; (3) Vycor glass column; (4) chiral conductive resin].

are +1 and −1, respectively, and proline and phenylalanine are neutral. The loading and reloading of Cu(II) in column 1 and 2 was controllable by applying 0.1 *M* $CuSO_4$ in 0.1 *M* $NH_4OH$ for the Cu(II) complex formation, and 0.05 *M* HCl was used to remove the Cu(II). Column 1 and 2 were regenerated after two consecutive operations.

### *2.4. Electroless separation of amino acids*

Initially, 20 μl of 0.03 *M* D,L-proline in the sodium acetate and $NH_4OH$ buffer at pH 7.0 was introduced into the triple column liquid chromatography system with no electric potential applied to column 1. This electroless separation experiment was repeated with racemic lysine and aspartic acid solutions to determine the performance of optical resolution in the absence of an electric field.

In order to corroborate the enantioseparation factor provided by the chiral cavities formed in the polypyrrole coating, another electroless separation experiment with L-proline methyl polystyrene free of polypyrrole coating was carried out. Columns 1 and 2 were packed with L-proline methyl polystyrene free

of polypyrrole coating where the 5 m*M* sodium acetate, 5 m*M* $NH_4OH$, and 1 m*M* EDTA buffer was used as the mobile phase.

### 2.5. Electrokinetic separation of amino acids

A mixture solution of 0.03 *M* D,L-lysine and 0.03 *M* D,L-aspartic acid in the same buffer was injected into the chromatography system where a potential difference of ±1.5 V was applied in column 1. After the D,L-lysine had been completely displaced, the polarity of the electric field in column 1 was reversed to displace the electrosorbed aspartic acid. This experiment was repeated with a racemic lysine–proline mixture and a aspartic acid–proline mixture. The electric field in column 1 was alternated in a way that charged column 1 adsorbed the ionic amino acids while the neutral proline molecules were eluting. The eluting amino acids were analyzed by using a refractive index detector (RI-4, Varian) and a polarimeter (Model 243B, Perkin-Elmer, Norwalk, CT, USA).

## 3. Results and discussion

### 3.1. Chiral separation of amino acids

#### 3.1.1. Batch experiment

The ligand exchange capacity of the imprinted PPy/L-proline methyl polystyrene composite was 0.31 meq. per g of resin (0.0357 g of D-proline per g of resin) when the exchanging ligand was D-proline. Provided that the maximum possible number of cavities were occupied by the D-proline, the number of accessible functional groups on the resin had decreased from 0.39 meq. $g^{-1}$ after the PPy coating. This was probably because the imprinted binding sites were occupied by the print molecules or because they were inaccessible to the amino acid solution. The adsorption isotherms of D- and L-proline on the imprinted adsorbent from the batch experiments are shown in Fig. 2. Both isotherms exhibited Langmuir-type adsorption profiles. The adsorbent has a stronger affinity to the D-isomer.

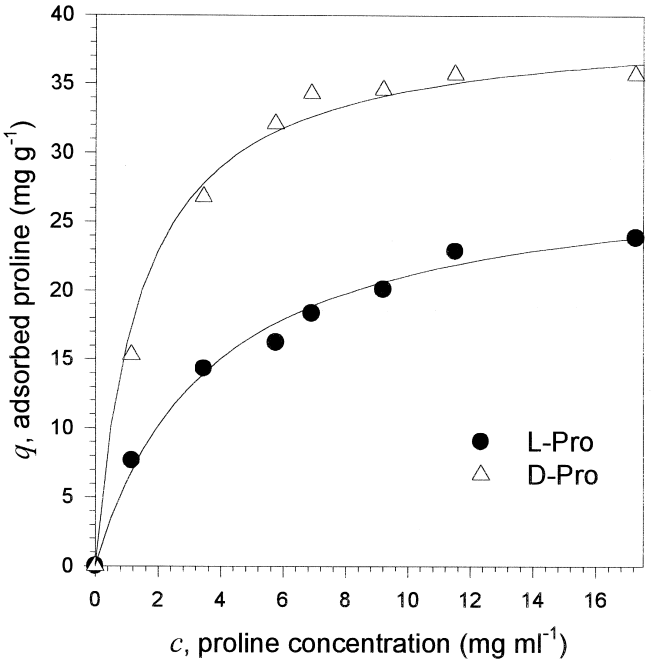


Fig. 2. Adsorption isotherms of D- and L-proline on the molecularly imprinted adsorbent at 25°C.

*3.1.2. Column chromatography*

A breakthrough test determined that the void volume of the triple column system was 1.09 ml ($t_{NR}$ = 4.36 min at 0.25 ml $min^{-1}$). The results of the electroless resolutions of racemic proline, phenylalanine, lysine, and aspartic acid are shown in Fig. 3. The enantioselectivity of the chromatography system with respect to D,L-proline was $\alpha_{D/L\text{-Pro}} = 1.91$. A partial resolution of lysine by this imprinted column was seen [see Fig. 3(c)] with a selectivity value of 1.22. The resolution of D,L-aspartic acid is shown in Fig. 3(d), of which the selectivity was $\alpha_{D/L\text{-Asp}} = 1.18$. A trend to lower selectivity values with increasing flexibility and decreasing size of side chain was observed. The chromatogram of D,L-phenylalanine in Fig. 3(b) does not show the separation. It is speculated that the chiral selectors inside the cavities were inaccessible to both the isomers due to the large side chain of phenylalanine. The molecular radii of the analytes play an important role in the extent of resolution by chiral cavities. Amino acids with smaller or larger side chains compared to that of the print molecule (D-proline) are expected to have less selectivities. Both enantiomers of smaller amino acids can enter the imprinted cavities and form Cu(II) complexes, while larger amino acids are unable to reach the enclosed Cu(II) ions. It is possible to efficiently separate a group of amino acids with larger or smaller side chains by choosing print molecules with appropriate molecular sizes.

The results of an independent experiment with L-proline methyl poly(styrene-*co*-divinylbenzene) free of polypyrrole showed less efficient separation performance as compared to that with polypyrrole-coated adsorbent. The enantioseparation factor for racemic proline was $\alpha_{D/L\text{-Pro}} = 1.05$.

*3.2. Electrokinetic and chiral separation of amino acids*

The polypyrrole-coated adsorbent pressed into a 400 nm thick film had an average resistance of 0.43 $\Omega\ \mu m^{-1}$. When a potential difference of 1.5 V was applied to column 1, the electric current across the porous Vycor tubing wall wetted with the sodium acetate buffer was in the 0.1–0.6 mA range.

Shown in Fig. 4(a) and (b) are the electrokinetic separations of racemic lysine and aspartic acid. The elution order of lysine and aspartic acid is altered depending upon the order of the polarity changes. The eluting amino acids in the first wave of two peaks were the ions deflected from charged column 1. These amino acids were resolved primarily by column 2. The amino acids in the second wave were initially adsorbed by the electrokinetic force in column 1 as well as by the Cu(II) chelate binding energy, then eluted by reversing the polarity of the electric field in column 1. A more efficient resolution was expected for the secondly eluting amino acids, because both columns 1 and 2 were employed for the resolution. In addition to, since the amino acids were adsorbed by coulombic attraction on column 1, the mass transfer resistance between the mobile and the stationary phases in column 1 was less significant as compared to conventional ion-exchange chromatography. As shown in Fig. 4(b), D,L-aspartic acid was not resolved showing that the enantioseparation factor contributed only by column 2 was not enough for the resolution of aspartic acid. Adversely, the selectivity value for D,L-aspartic acid determined from the elution profile in the second wave [Fig. 4(a)] was improved. More comprehensive sorption equilibria in column 1 were possible due to less mass transfer resistance on the column surface.

In Fig. 4(c) and (d), the chromatograms of racemic lysine–proline and aspartic acid–proline mixtures are shown. The resolved lysine and aspartic acid could be displaced by changing the polarity of the electroconductive column. The sample amino acid mixtures were separated according to their charge characteristics as well as optical isomerisms.

## 4. Conclusions

This paper presents preliminary experimental results of electrokinetic and, simultaneously, chiral separation of amino acid mixtures. The proposed chromatographic separation process utilizes two driving forces; coulombic interaction by an electric field, and chiral recognition by the molecularly imprinted templates. The imprinted templates also exhibited a size exclusion effect which can be controlled by varying the size or the shape of the print molecule.

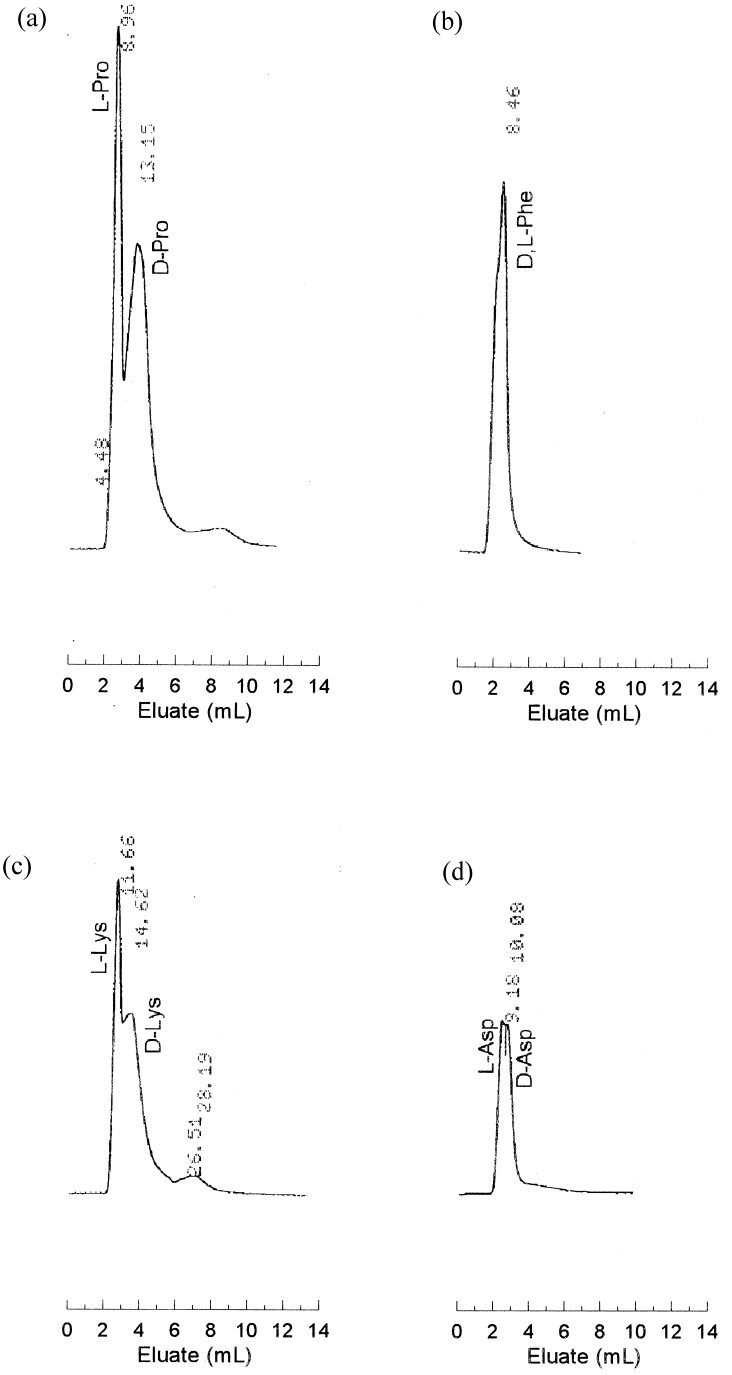


Fig. 3. Optical resolution of (a) D,L-proline, (b) D,L-phenylalanine, (c) D,L-lysine, and (d) D,L-aspartic acid using a molecularly imprinted ligand exchange column. A 5 m*M* $NH_4OH$ and 5 m*M* sodium acetate in 1 m*M* EDTA solution at pH 7.0 was the mobile phase flowing at 0.25 ml $min^{-1}$.

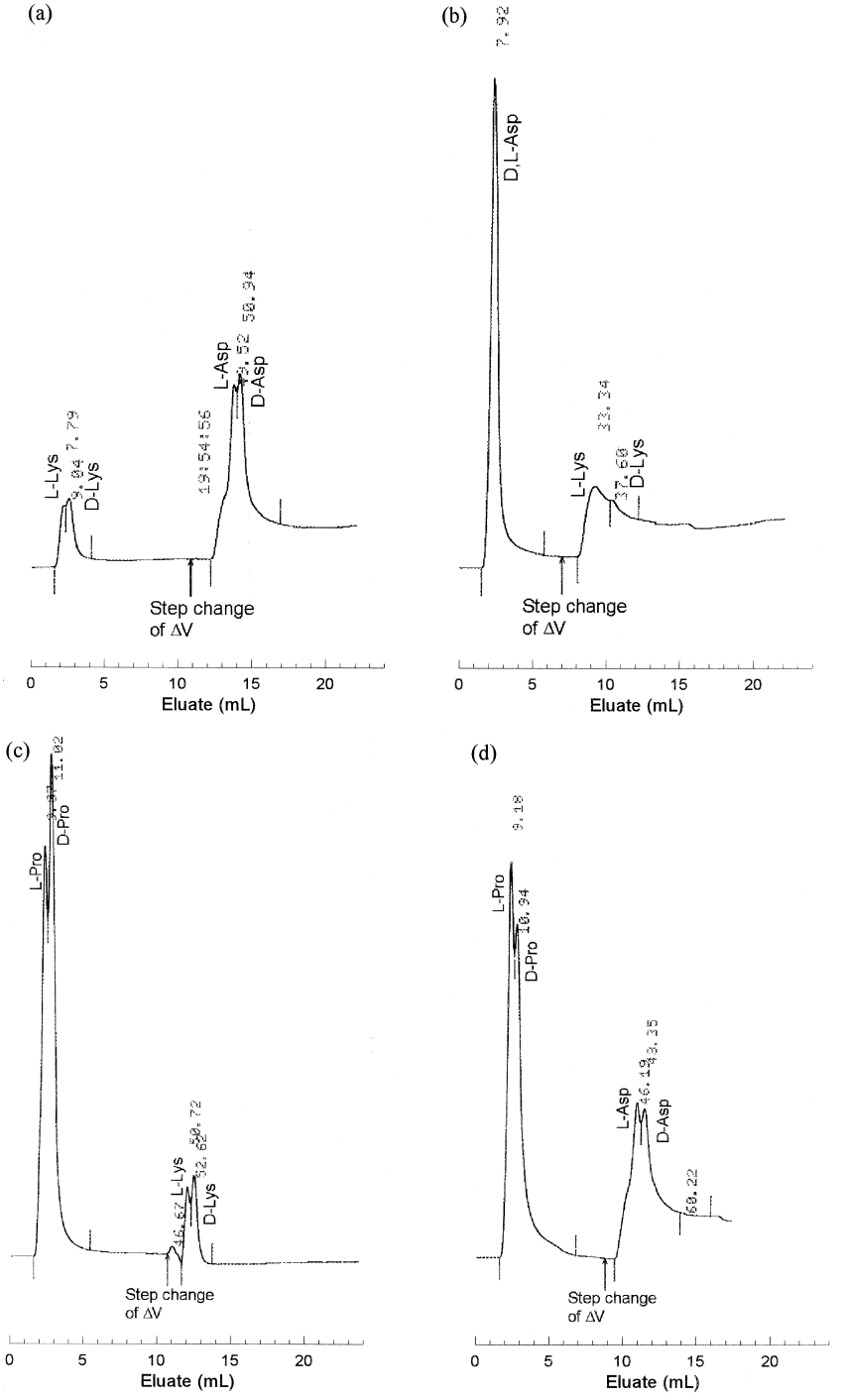


Fig. 4. Dynamic elution of racemic amino acid mixtures. The elution rate is 0.25 ml $min^{-1}$. The step voltage wave form in column 1 is: (a) from −1.5 V to +1.5 V for the displacement of adsorbed D,L-lysine, (b) from +1.5 to −1.5 V for the opposite sequence, (c) from −1.5 V to +1.5 V for the displacement D,L-lysine, and (d) from +1.5 V to −1.5 V for the displacement of electrosorbed D,L-aspartic acid.

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