Asian Journal of Chemistry; Vol. 28, No. 11 (2016), 2411-2414

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19993

Zwitterionic Ion Chromatography of Dansyl Amino Acids with 4-Vinylbenzyl Dimethyl Ammonio Pentanesulfonate as Stationary Phase

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Received: 25 March 2016; *Accepted*: 1 July 2016; *Published online*: 10 August 2016; AJC-18008

A high-capacity (488 μeq g^{-1}) zwitterionic stationary phase was synthesized by attachment of zwitterionic precursor to polystyrene-divinylbenzene particles by graft reaction. Zwitterionic ion chromatography for the determination of dansyl-L-asparagine (D-L-Asp), dansyl-glycine (D-L-Gly), dansyl-L-valine (D-L-Val) and dansyl-L-leucine (D-L-Leu) was developed. Chromatographic characteristics of the stationary phase are investigated by separation of dansyl-amino acids using buffer eluent with UV/visible detection. It could be demonstrated that dansyl amino acids separation on zwitterionic stationary phase are mainly driven by a cation exchange mechanism. The sulfobetaine column already mentioned exhibited the difference as compared to the commercial columns.

Keywords: Amino acid, Ion exchange, Zwitterionic stationary phase, Sulfobetaine, Capacity.

INTRODUCTION

Zwitterionic ion chromatography (ZIC) is an alternative form of ion chromatography (IC), which uses zwitterionic ion chromatography columns for cation and anion separations [1]. The notion combines both cationic and anionic groups in a one molecule on the column in order to improve the ion exchange selectivity [2,3]. This form of ion chromatography was initially proposed in 1993 [4]. Amino acid analysis and detection has been for decades a challenging task. The classical methods for analysis dansyl-amino acids for long time have been RP-LC and ion exchange chromatography with fluorescence or UV absorbance detection. The drawback of these methods required post-/pre-columns derivatization of the solutes [5]. Consequently, in previous years various separation methods have been developed to accomplish betterment in detection and separation. Therefore, one of the methods promising and rapidly growing is zwitterionic ion chromatography [6,7].

The number studies of zwitterionic ion chromatography retention mode, the separation mechanism still remaim unresolved until today. There are two mechanisms engaged in zwitterionic ion chromatography. Firstly, a donnan membrane mechanism based on both the chaotropic interaction and a shield effect [8,9]. Secondly, a binary electrical double layer model (BEDL) [10,11]. The main focus of our work was the separation of D-amino acids (Fig. 1) and retention characteristics in zwitterionic ion chromatography-mode with eluents containing eluent ions but no organic modifiers on a novel PS/DVB stationary phase, covalently attached to 4-vinylbenzyl-dimethylammonio pentanesulfonate. Sodium acetate eluent was investigated and showed interesting separation mechanisms.

EXPERIMENTAL

The experimental work was performed with a modular HPLC system (Merck-Hitachi). For pumping the eluent a L-6200 pump was used. The injection valves (20 μL injection loop) as well as the column heater were located in the separation center and a UV/visible detector (L-4200) was used. The wavelength for UV detection was 220 nm. The X-ray fluorescence (XRF) measurements for capacity determination were performed on an ARL Optim'X (Thermo Fisher, Dreieich, Germany). CHNS elemental analysis was performed on a Vario Micro cube from Elementar (Hanau, Germany). The pH measurements were conducted on pH 7110 (WTW).

Reagents for the synthesis of the zwitterionic stationary phase were used in highest available purity. 1,5-dibromopentane (97 %) was obtained from Aldrich. Sodium metabisulfite (≥ 98 %) was purchased from Merck. 4-vinylbenzyl-N,N-dimethyl amine (90 %) was purchased from Acros Organics. The core material consisted of highly cross-linked macro porous PS/DVB copolymer. The crosslinking degree was 55 %, particle size was 4.6 μm. ZIC-pHILIC and ZIC-

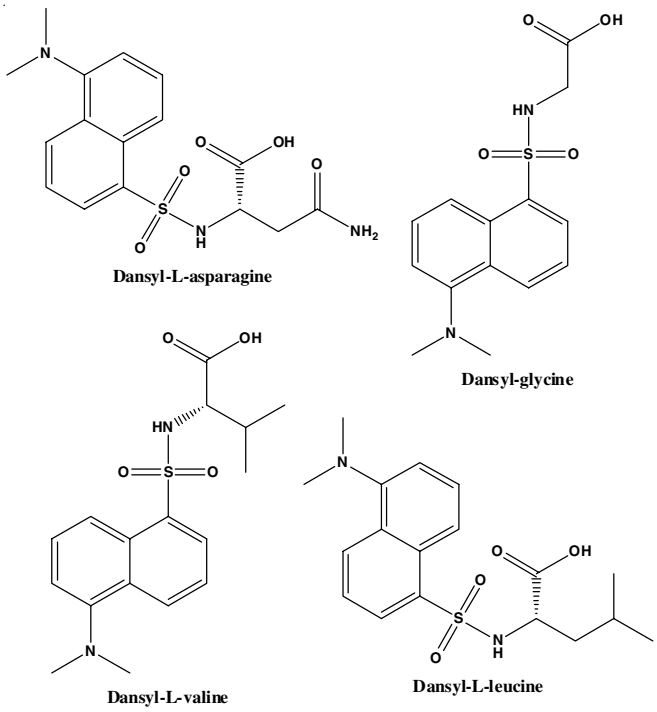

Fig. 1. Structure of dansyl amino acids

HILIC columns were obtained from Merck SeQuant (100 mm × 4.6 mm I.D.). The ZIC-HILIC and ZIC-pHILIC columns have either silica or methacrylate cores and the commercial exchangers have three methylene groups between the charged functional groups. Dansyl-L-asparagine, dansyl-glycine, dansyl-L-valine and were purchased from Sigma-Aldrich. Acetic acid was obtained from Carl Roth (Karlsruhe, Germany). Sodium acetate was obtained from J.T. Baker (Deventeer, Netherlands). Distilled deionized water (Milli-Q, Millipore) was used for sample preparation as well as for eluents and rinsing of the system.

Preparation of zwitterionic stationary phase ZIC-5: The zwitterionic molecule having five methylene groups between inner quaternary amines and outer sulfonic acids (ZIC-5) was prepared according to a procedure which adopted from previous work [12]. Nucleophilic substitution reactions between a monomeric spacer and tertiary amines were used for the synthesis of this molecule. 4-Vinylbenzyl-dimethyl-ammonio pentanesulfonate was prepared *via* two steps: Firstly, the synthesis of 5-bromopentane-1-sulfonate by the reaction of 1,5-dibromopentane with sodium metabisulfite [13]. Secondly, the reaction of 5-bromopentane-1-sulfonate with 4-vinylbenzyl-N,N-dimethylamine to give 4-vinylbenzyl-dimethylammonio pentanesulfonate (Fig. 2). Functionalization of the PS/DVB is implemented by a grafting reaction following a preparation are carried out according to the Raskop *et al.* [14] by configuring covalent bonds between the zwitterionic monomer to the polymeric particles (Fig. 2). The stationary phase is packed using PEEK column (100 mm × 4 mm ID).

Determination of capacities: Capacity of zwitterionic exchanger was determined by detecting the sulfur contents *via* combustion elemental analysis CHNS (Elementar, Germany) and XRF (Thermo Fisher, USA).

RESULTS AND DISCUSSION

Determination of the zwitterionic column capacity: The zwitterionic material exhibits inter- and intra-molecular interactions between the charged sites in columns [15,16] and therefore, the classical methods cannot be implemented for determining capacities. The averaged capacity (488 μeq g^{-1}) of the zwitterionic column calculated from the nitrogen and sulfur contents from XRF and elemental analysis.

Separation of amino acids: Amino acids separations under ZIC conditions are investigated. The four amino acids are separated (Fig. 3) using sodium acetate eluents and the zwitterionic stationary phases (Z IC-5, ZIC-HILIC and ZIC-pHILIC). The highest retention times can be observed for ZIC-5 columns and the commercial columns showed the lowest retention time. It should be noted that the differences in the chromatograms of the separations of four amino acid due to the spacer length between the charges in stationary phases. The mobile phase conditions are changed systematically by varying pH and the concentration of eluent, to get a clue into the characteristics and separation mechanism of ZIC column.

Effect of eluent concentration on retention of amino acids: In a previous study [1,15,16], the retention times for

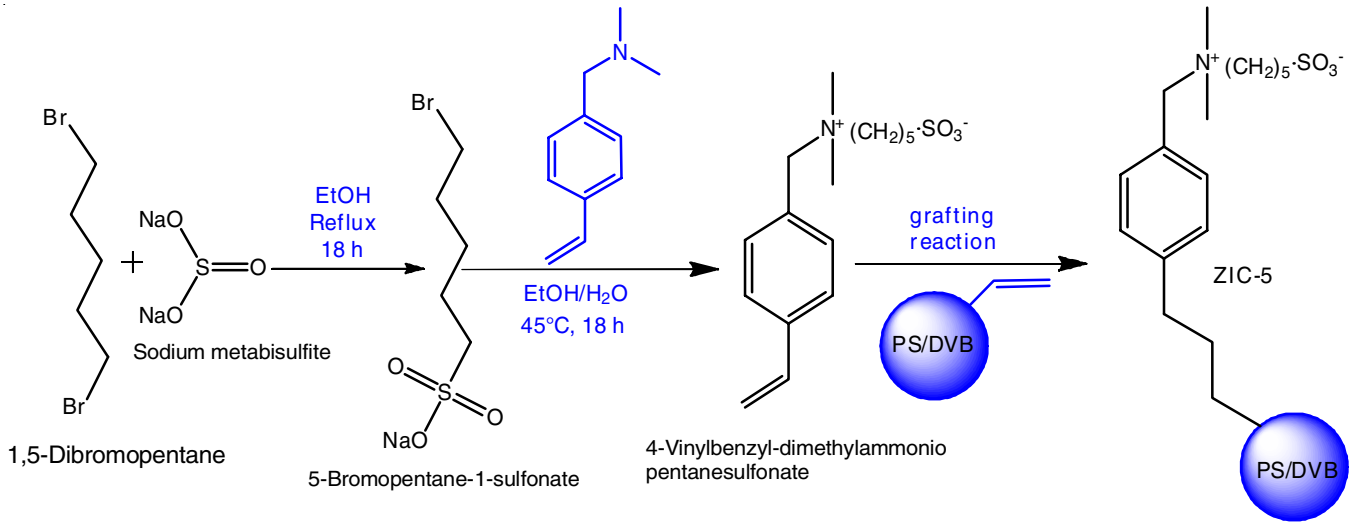

Fig. 2. Schematic reaction sequence of the preparation of ZIC-5 column

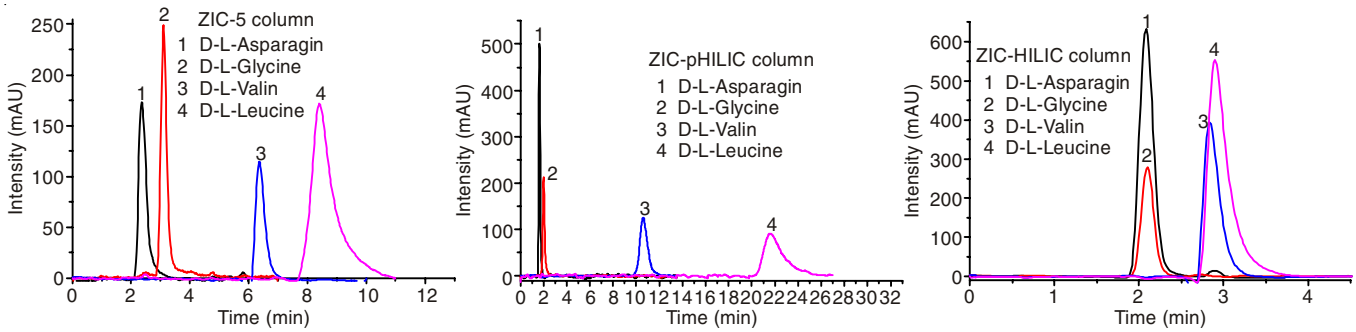

Fig. 3. Separation of amino acids. Eluent: (10 mM sodium acetate, pH 4.75; UV detection at 220 nm; flow rate: 1.0 mL/min; temperature: 318K; analyte concentration: 10 mg/kg

the anion separations increased with increasing eluent concentration using ZIC-mode. Thereby, the separation mechanism follows a binary electrical double layer mechanism [10,11]. Fig. 4 shows a different picture, the retention factor of D-amino acids decreased, when the ionic strength was increased from 10 to 60 mM. This impact has been noted for all ZIC columns. The isoelectric points of the D-amino acids are between 5.31 and 5.98 (Table-1). Under the selected circumstances, the investigated D-amino acids must be in a more cationic form.

TABLE-1
PHYSICO-CHEMICAL PROPERTIES
OF THE AMINO ACIDS [Ref. 18]

Compound	Isoelectric point	pK_a
D-L-asparagine	5.31	4.65 (NH_2); 3.23 (COOH)
D-glycine	5.97	4.55 (NH_2); 3.24 (COOH)
D-L-valine	5.96	4.66 (NH_2); 3.40 (COOH)
D-L-leucine	5.98	4.66 (NH_2); 3.47 (COOH)

The commercial columns ZIC-HILIC and ZIC-pHILIC exhibit low retention factors in comparison to ZIC-5 column. The slope log retention factor *versus* log eluent concentration (Fig. 3) seems to be like the slope measured for classical ion exchange columns [17]. Eqn. 1 describe the relation between k′ for the analyte and ionic strength in the mobile phase, $E_m^{z_e}$:

$$\log k' = C - \frac{z_s}{z_e} \log E_m^{z_e} \quad (1)$$

where, z_s the charge analyte, z_e the charge eluent, C is the constant and the slope ($-z_s/z_e$). Accordingly, ZIC-5 column exhibits cation exchange mechanism behaviour (Table-2). This behaviour is interesting due to ZIC columns show a different separation mechanisms [9,10].

The reasons for this difference in behaviour of zwitterionic columns (ZIC-5, ZIC-HILIC and ZIC-pHILIC) are: firstly, the difference in the chain length between the charged functional groups. Secondly, the capacity of ZIC-5 column was 488 μeq g^{-1} while for ZIC-HILIC and ZIC-pHILIC columns were 186 and 201 μeq g^{-1}, respectively [16]. Increasing the capacity lead to an increased retention time of the dansyl amino acids. ZIC-5 (five methylene groups) column have more chain length than ZIC-HILIC and ZIC-pHILIC (three methylene groups) columns. Therefore, the ZIC-5 molecules on PS/DVB surface remain more flexible.

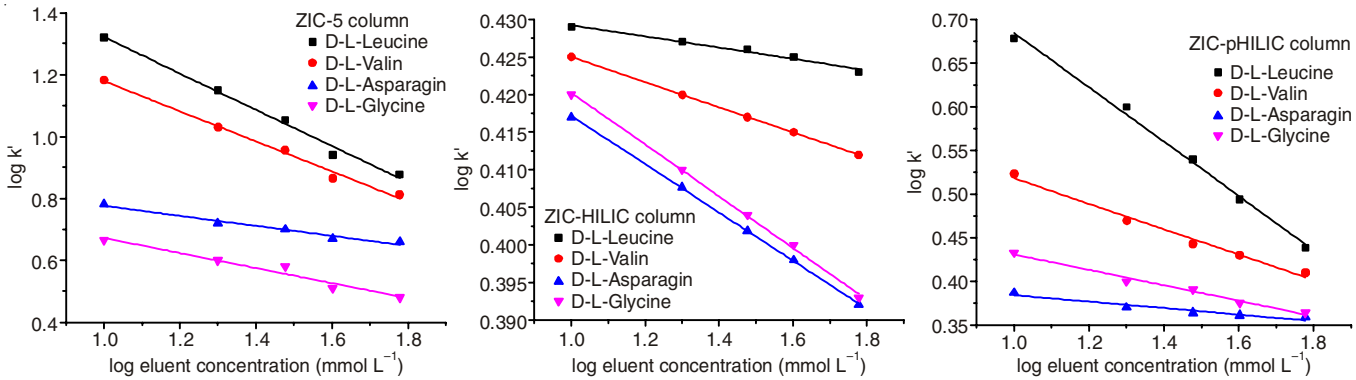

Fig. 4. Influence of buffer strength on the D-amino acids retention. Eluent: sodium acetate buffer (pH 4.75); flow rate: 1.0 mL/min, using ZIC-5, ZIC-pHILIC and ZIC-HILIC columns

TABLE-2
SLOPE VALUES FOR log k' *vs.* log ELUENT CONCENTRATION DEPENDENCIES
FOR ZIC-5, ZIC-HILIC AND ZIC-pHILIC COLUMNS IN THE AMINO ACIDS

Compound/Column		D-L-asparagine	D-glycine	D-L-valine	D-L-leucine
ZIC-5	Slope	-0.1618	-0.2434	-0.4870	-0.5860
ZIC-HILIC	Slope	-0.0320	-0.0343	-0.0166	-0.0074
ZIC-pHILIC	Slope	-0.0365	-0.0885	-0.1458	-0.3109

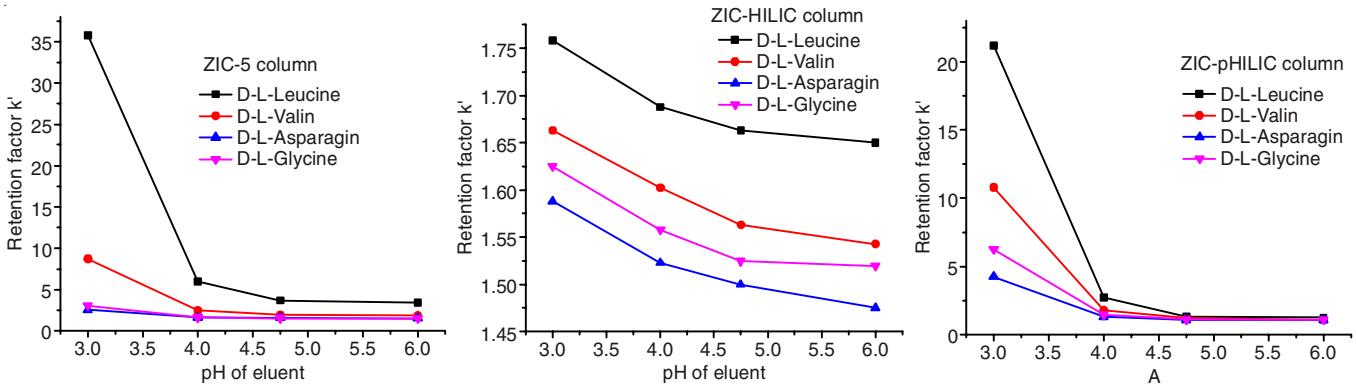

Fig. 5. Influence of eluent pH on D-amino acides retention. Eluent: sodium acetate buffer (10 mM); flow rate: 1.0 mL/min, using ZIC-5, ZIC-HILIC and ZIC-pHILIC columns

Effect of eluent pH on retention of amino acids: A reduction in pH of buffer leads to increases cation exchange capacity of zwitterionic molecules. The influence of eluent pH was examined for different ZIC columns over a pH interval 3 to 6 at constant 10 mM sodium acetate buffer. At a high pH of eluent the retention factors of D-amino acids decreased and increasing with decreasing pH (Fig. 5). Accordingly, all of the investigated D-amino acids must be exist in cationic state, when decreasing the eluent pH and, therefore, increased interactions of D-amino acids with the ZIC columns. In one word, the dominant separation mechanism for the D-amino acid separations using ZIC-5 column are the cation exchange [15].

Conclusion

This article shows how the four ionized D-amino acids interact with a new zwitterionic stationary phase (ZIC-5). It was found, that the ZIC-5 material shows higher interaction with D-amino acids in comparison to commercial columns. The reason may be due to be a geometrical alignment of sulfobetaine groups in the ZIC-5 column as they exhibit the highest D-amino acid retention. The cation exchange is the dominant separation mechanism for D-amino acid separations under ZIC-mode. The ZIC type material being until now only known to suited for anion separation, but we have proven the suitability of this type for cation separation.

ACKNOWLEDGEMENTS

The authors thanks Prof. Dr. Andreas Seubert for his support of the research project. Routine analytical service from Philipps-University Marburg is acknowledged for performing elemental analysis.

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