ICP Spectrometric–Vis Separation of Cerium (IV)–Desferal Complex Using 4-Vinylbenzyl-Dimethylammonio Pentanesulfonate Zwitterionic Stationary Phase

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Abstract

A new covalently bonded sulfobetaine stationary phase with an outer positive charge and an inner negative charge was synthesized by attachment of zwitterionic molecule to PS/DVB particles by graft polymerization. Desferal (DFOM) is a chelating agent which forms complexes with multiple charged metal ions of biological importance. We could demonstrate that the zwitterionic stationary phase is able to separate the Ce(IV)-DFOM complex by IC-ICP-AES. It could be demonstrated that metal-DFOM separations on zwitterionic stationary phase are mainly driven by a cation exchange mechanism. This is a behavior never before observed using zwitterionic stationary phase. In spite of numerous publications in the literature which confirm that zwitterionic type materials are usually well suited for anion separation, no investigation had been carried out to separate cationic organic compounds. The sulfobetaine column already mentioned exhibited the difference as compared to the commercially ZIC-HILIC and ZIC-pHILIC columns.

Keywords: Zwitterion chromatography/ Sulfobetaine stationary phases/ Desferal/ Retention mechanism/ ion exchange.

1. Introduction

The drug desferal also called desferrioxamine (DFO, a pharmaceutical compound), chemically 30 – amino-3, 14, 25-trihydroxy-3, 9, 14, 20, 25 pentaazatriacontane -2, 10, 13, 21, 24- pentone methanesulphonate Fig.(1), is deemed to be a supreme for the clinical treatment of several diseases specially for those related to the metal intoxication in human subjects, for example, thalassemia [1] (iron over-load), Alzheimer [2] and renal disorders (aluminum over-load) [3].

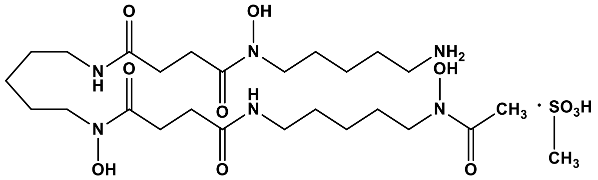


Fig.(1): The structure of desferrioxamine mesylate (DFOM).

DFOM is a strong chelator for multiple charged metal ions of biological importance (Fe^{3+}, Al^{3+}). DFOM is trihydroxamic ligand containing six oxygen-donors (three bidentate metals) and are capable of complete octahedral coordination to form a highly stable $M^{3+}L$ complex [4, 5]. DFOM has four protonation constants, the first three constants relate to the hydroxamate groups and the fourth to the terminal amino group. DFOM is in the physiological pH range which most of the time occurs in the fully protonated form of H_4DFOM. Al-Ayash et al. [6-8] developed indirect analysis of the drug DFOM by using UV-Vis spectrophotometric and ETAAS combined with zirconium-coated graphite tube. They suggested methods are based on the reaction of the drug with Au (III) and V (V) as pairing agents.

Despite the availability of numerous studies in desferal analysis, no investigation has been carried out for the cerium (IV)–Desferal complex Hydrophilic interaction liquid chromatography (HILIC) performed in zwitterionic stationary phases (ZIC-HILIC) is an upcoming separation technique with rapidly increasing importance. It is suitable for the separation of hydrophilic and ionic substances. The separation mechanisms occurring in the ZIC-HILIC is more complicated. Despite the numerous studies about the ZIC-HILIC retention mode, the mechanism has not been fully investigated and there are no detailed mechanistic concepts in the literature. The ZIC-HILIC mode offers a new mixed-mode HPLC based on to the electrostatic interaction with the positively and negatively charged functional groups on the ZIC-stationary phase and hydrophilic interaction. Moreover, the multipoint interactions such as hydrophilic interaction, ion-exchange interactions, hydrophobic interaction and dipole–dipole interactions contribute to the retention of analytes in ZIC-HILIC mode. HILIC is applying to the separation pharmaceutical compounds [9-11], carbohydrates [12, 13], peptides [14-17] and saccharides [18].

It will be interesting to see if the metal–complexes are also separated using the ZIC-HILIC-columns. Recently, Rasheed et al. [19] had studied the separation of the Fe(III)-DFOM and Al(III)-DFOM complexes by ZIC-ICP-AES. Consequently, the main focus of work was into the separation of Ce (IV)-DFOM and retention characteristics in a novel PS/DVB stationary phase, covalently attached to 4-vinylbenzyl-dimethylammonio pentanesulfonate.

2. Experimental

2.1. Instrumentation

All spectral and absorbance measurements were carried out on a Shimadzu UV–Visible 1800 a digital double–beam recording spectrophotometer using 1-cm silica cell. A Metrohm 790 Personal ion chromatograph (Metrohm AG, Herisau, Switzerland), an injection valve (10-µl injection loop) as well as the column heater were used. The column outlet was directly connected to the nebulizer of the ICP-AES. The element specific detection was utilized by a Spectroflame P (Spectro Analytical Instruments GmbH, Kleve, Germany) with a cross-flow nebulizer and a Scott spray chamber. The emission line was 393.373 nm (cerium). The pH measurements were conducted on pH100 (VWR). To carry out Ce(IV)-DFOM separation, the aqueous eluent phase and the organic modifier were mixed (80:20) before they passed to the pump. The 20% of the organic modifier was used in all measurements in order to avoid extinguishment of the plasma. The pump for pumping the aqueous phase was made of PEEK. The eluent passed to a T-shaped connection, which was linked with a peristaltic pump on-line to add water in order to dilute the mobile phase. We used a pulsation dampener between the pump and the injection valve.

2.2. Reagents

Cerium (IV) sulfate tetrahydrate and Deferoxamine mesylate salt were purchased from Sigma. 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 (surface area (S) = 1073 m^2/g, mean pore diameter (φ_{50}) = 47 Å, specific pore volume (V_{Ps}) = 1.27 mL/g, total porosity (ε_T)=70.5%). ZIC-pHILIC and ZIC-HILIC columns were obtained from Merck SeQuant (100mm × 4.6mm I.D.). The ZIC-HILIC and ZIC-pHILIC columns have either silica or methacrylate cores and the commercially exchangers have three methylene groups between the charged functional groups. The cation exchange columns was self-made via a grafing reaction adopted from patent [20] using PEEK columns (100 mm × 4 mm I.D.). Acetonitrile for HPLC was obtained from Sigma-Aldrich. Acetic acid was obtained from Carl Roth (Karlsruhe, Germany). Sodium acetate was obtained from J.T.Baker (Deventeer, Netherlands). Distilled deionised water (Milli-Q, Millipore) was used for sample preparation as well as for eluents and rinsing of the system.

2.3. 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 [21]. Nucleophilic substitution reactions between a mon meric spacer and tertiary amines were used for the synthesis of this molecule.

4-vinylbenzyl dimethylammonio pentanesulfonate was prepared via two steps: Firstly, the synthesis of 5-bromopentane-1-sulfonate by the reaction of 1,5-Dibromopentane with sodium metabisulfite [22]. 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 developed Raskop et al. [20] 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 I.D.).

2.4. Determination of capacities

Capacity of zwitterionic exchanger was determined by detecting the sulfur contents via combustion elemental analysis CHNS (vario Micro cube, Elementar, Hanau Germany) and x-ray fluorescence XRF (ARL Optim’X, Thermo Fisher, Waltham, MA, USA) on the sulfur K $\alpha_{1,2}$ line at 5.3731 Å using the PET crystal.

3. Results and Discussion

3.1 Absorption spectra

3.1.1 Desferrioxamine mesylate

Fig.(3b) shows the absorption spectra of drug. The maximum absorption was at 213 nm of 100 µg mL^{-1} DFOM.

3.1.2 Cerium (IV) sulfate tetrahydrate

The absorption spectra of 20 µg mL^{-1} cerium (IV) and a maximum absorption was at (274 nm) as shown in Fig.(3a).

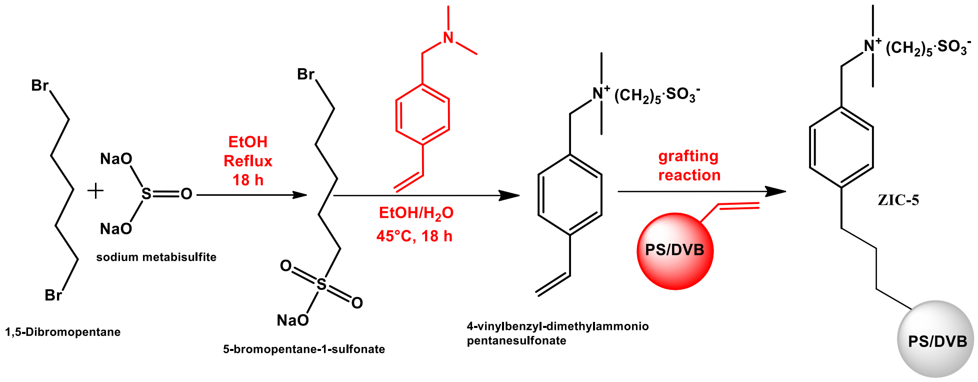


Fig.(2): Schematic reaction sequence of the preparation of ZIC-5 column.

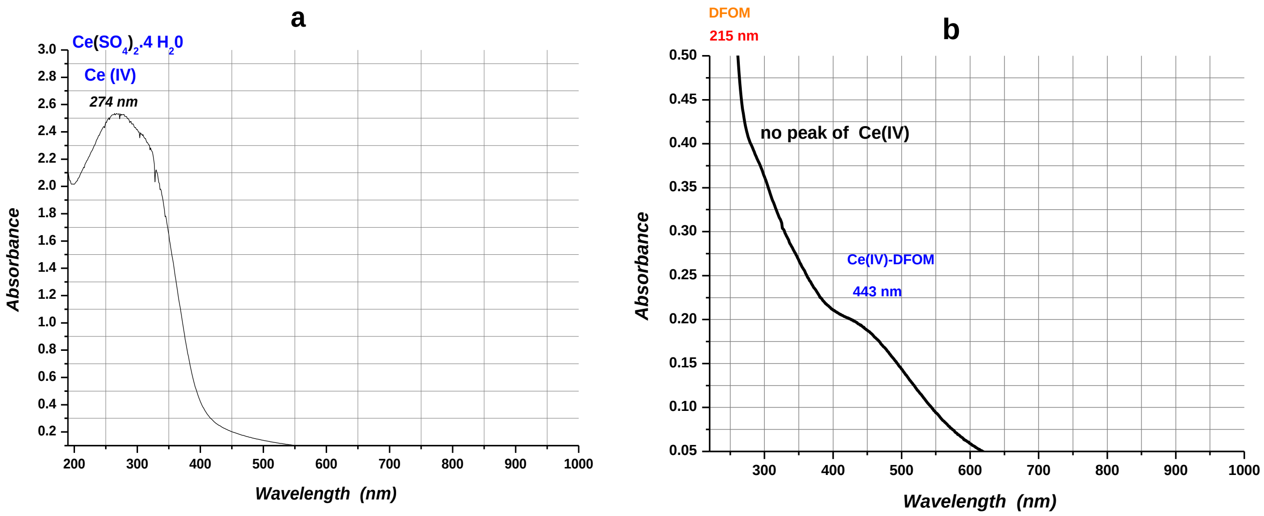


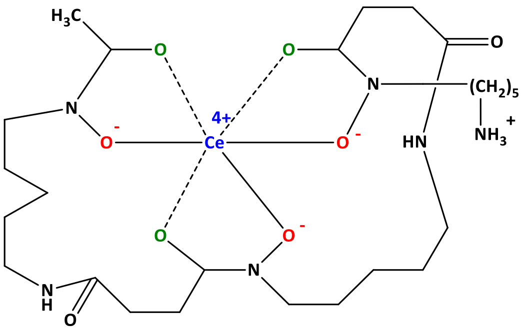
Fig.(3): Absorption spectrum
(a) Cerium (IV) sulfate tetrahydrate.
(b) Desferrioxamine mesylate and cerium (IV)-DFOM complex.

3.1.3 Orange complex of DFOM with Cerium (IV)

The absorption spectrum of complex (100 µg mL^{-1} DFOM-20 µg mL^{-1} Ce (IV)) in pH= 6 was 443 nm Fig.(3b).

3.1.4 The molar ratio of ligand (L) to metal (M)

The molar-ratio method was used to determine the stoichiometry of the complex. **A** 1: 1 stoichiometric ratio of H_4DFB^+ of the Ce $^{+4}$ ion was found as shown in Fig(4).



Ce(IV)-$(DFOH)^+$

Fig.(4): Probable chemical structure of the Ce (IV)-DFOM complex.

3.2 Determination of the zwitterionic exchanger capacity

The zwitterionic material shows intramolecular/ intermolecular saturation between positively and negatively charged in stationary phases [19,23] and, therefore, dynamic methods of determining capacities in chromatography cannot be implemented. The averaged capacity (488 µeq g^{-1}) of the zwitterionic exchanger calculated from the sulfur content from XRF and elemental analysis.

3.3. Separation of Ce(IV)–DFOM complex

The Ce(IV)-DFOM complex separation is investigated, using sodium acetate eluents with 20% acetonitrile content. Retention of this complex was observed for ZIC-5 stationary phase. In accordance with the a previous study [149], Deferoxamine has four protons, one from the protonated a terminal primary amine group and three from the hydroxamic acid group $(H4\text{-}DFOM)^+$. Therefore, Ce(IV)-DFOM complex reaction is illustrated in Equations 1:

$$H_4 - DFO^+ + Ce^{4+} \rightarrow 3H^+ + Ce\,(H - DFOM)^+ \quad \text{....................................} (1)$$

The ZIC-5 column showed the highest retention time ($t_{R,\ Ce}$ = 22.7), and the ZIC-pHILIC column (commercially column) showed the lowest retention time ($t_{R,\ Ce}$ = 3.5) of Ce(III)-DFOM complex. The chromatogram of the Ce(IV)-DFOM complex is shown in Fig.(5).

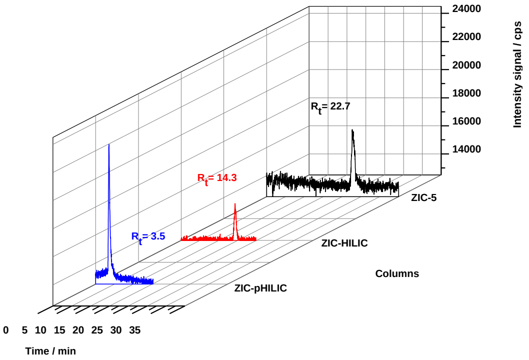


Fig.(5): Chromatograms for the separations of Ce(IV)-DFOM complex on ZIC-5, ZIC-HILIC and ZIC-pHILIC columns. Eluent: acetonitrile/sodium acetate buffer (pH 5.5, 40 mM) 20:80; flow rate: 0.5 ml/min; detection: ICP-AES at 393.373 nm, 318 k.

Eluent conditions are changed methodically by starting with a variation of acetonitrile content, concentration and pH of eluent, in order to get a closer view into the properties of the individual zwitterionic stationary phases and thus about the separation mechanism.

3.3.1. The influence of buffer strength on the retention of Ce(IV)-DFOM complex

The first step of eluent composition variation was the concentration of the sodium acetate buffer while holding the eluent pH and acetonitrile fraction constant. The influence of acetate concentration on the retention was investigated by varying sodium acetate concentration from 20 to 80 mM. The retention factors of Ce(IV)-DFOM complex decreased with an increasing buffer concentration. The zwitterionic column-ZIC-5 as well as the examined commercial columns ZIC-HILIC and ZIC-pHILIC show the same behavior. It should be noted the investigated Ce(IV)-DFOM complex must be present in a more cationic state [24-26]. The slope of the log-log-plots for changing eluent ionic strength of sodium acetate Fig.(6a) looked like the plots observed for a pure ion exchange mechanism [27]. Therefore, the ion-exchange interactions with the ZIC-5 exchanger contributed greatly to the retention of the Ce(IV)-DFOM complex [28]. The slope of the plot of log retention factor versus log buffer concentration Fig.(6b) in eluent is only 0.06, which is much smaller than we can normally find in standard ion exchange chromatography [27]. Therefore, the separation mechanism of Ce (IV)-DFOM complex by commercial columns ZIC-HILIC and ZIC-pHILIC can be explained by the electrostatic interaction.

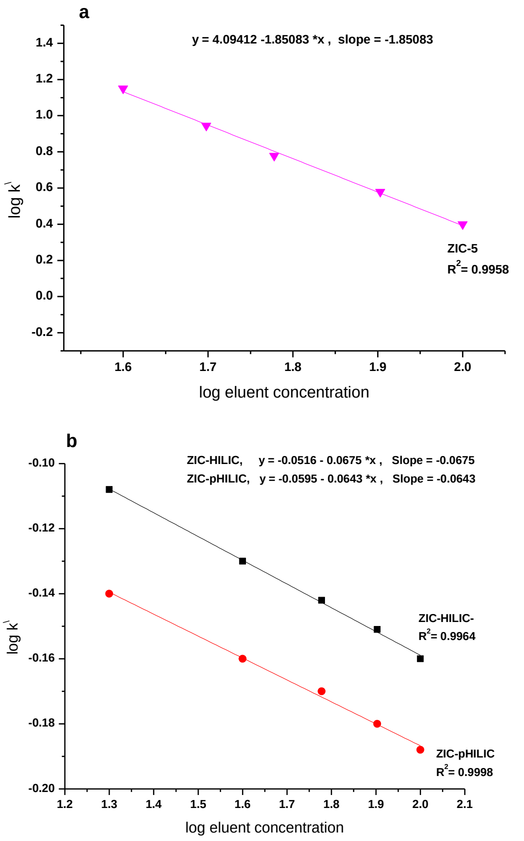


Fig.(6): Influence of buffer strength on the Ce(IV) DFOM complex retention. Eluent: acetonitrile/sodium acetate buffer (pH 5.5) 20:80; flow rate: 0.5 ml/min, using ZIC-5, ZIC-HILIC and ZIC-pHILIC columns.

The upcoming question now is what the true separation mechanism is. Because of the simultaneous variation of anion and cation concentrations, it is impossible to judge if it is anionic, cationic or mixed mode ion exchange retention. For a deeper investigation of the retention mechanism, a cation exchanger Fig.(7) based on the same core material and using the same grafting reaction [20] has been used. Accordingly, the separation of the positively charged of Ce(IV)-DFOM complex relied on the cation exchange with the ZIC-5 stationary phase. The reason for this difference in behavior of zwitterionic columns (ZIC-5, ZIC-HILIC and ZIC-pHILIC) toward varying buffer strength of the eluent is due to the difference in the capacity of the materials and the chain length between the charged functional groups. The capacity of ZIC-5 column was 488 μeq g^{-1} while the capacities of ZIC-HILIC and ZIC-pHILIC columns were 186 and 201 μeq g^{-1}, respectively [23]. Increasing the capacity means increasing the amount of functional groups on the surface of the stationary phase, which leads in this case to an increased retention of the Ce(IV)-DFOM complex. 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.

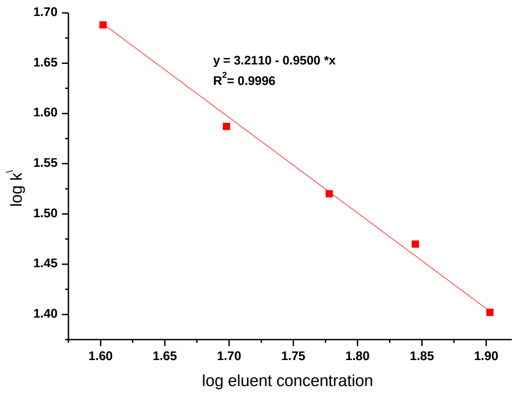


Fig.(7): Influence of eluent concentration on the Ce(IV)-DFOM complex. Eluent: acetonitrile/sodium acetate buffer (pH 5.5) 20:80; flow rate: 0.5 ml/min, using cation exchange column.

3.3.2. The influence of eluent pH on the retention of Ce(IV)-DFOM complex

After having exhibited the influence of the eluent concentration for the Ce(IV)-DFOM complex separations, the next variation in eluent composition is a variation of eluent pH. The pH of the eluent plays an important role for the retention of the metal-DFOM complexes by affecting metal-DFOM complexes ionization [19]. The influence of pH was investigated over the range from pH 4 to pH 6 with 40 mM sodium acetate and constant acetonitrile fraction of 20% Fig.(8). The retention of the Ce(IV)-DFOM complex with their positive charge decreased on ZIC-5, ZIC-HILIC and ZIC-pHILIC columns when the pH increased in the range between 4 to 6. This can be attributed to a stronger protonation of the Ce(IV)-DFOM complex. In other words, a decreased pH of the eluent leads to a more positive charged Ce(IV)-DFOM complex. Subsequently, more cation exchange interaction with zwitterionic surface takes place. In sum, it could be proved that cation exchange is the predominant separation mechanism for the metal-DFOM complex separations using ZIC-5 column [19].

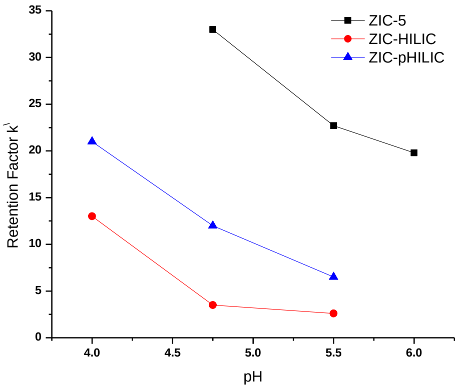


Fig.(8): Influence of eluent pH on Ce(IV)-DFOM retention. Eluent: acetonitrile/ sodium acetate buffer (40 mM) 20:80; flow rate: 0.5 ml/min, using ZIC-5, ZIC-HILIC and ZIC-pHILIC columns.

4. Conclusion

Zwitterionic stationary phase with five methylene groups between the charged groups was prepared on highly porous divinylbenzene polymers allows the investigation of the separation of metal-DFOM complex. The current study confirmed the ability of ZIC-HILIC columns to separate metal-complexes. ZIC on-line coupled to ICP-AES is an appropriate tool for the investigation of metal binding of DFOM. It was found, that the material shows higher interaction with Ce(IV)-DFOM complex and different behavior in the separation mechanism in comparison to commercially columns ZIC-HILIC and ZIC-pHILIC. The reason for this must be the chain length between charged groups and capacity of columns. The PS/DVB used as core material can play an important role in the retention of Ce(IV)-DFOM complex in comparison to silica or methacrylate core materials.

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الخلاصة

تم تحضير مبادل zwitterionic جديد من خلال تحضير مونمر zwitterionic ذو شحنه موجبه خارجيه وشحنه سالبه داخليه, بعد ذلك ربط المونمر بأصره تساهميه مع البوليمر PS/DVB بأستخدام طريقه graft polymerization. ان المركب الدوائي دسفيرال هو عامل كلابي يكون معقدات مع أيونات متعدده الشحنه ذو أهميه بايولوجيه. لقد برهنت ان المبادل zwitterionic له القدره على فصل المعقد Ce(IV)-DFOM بأستخدام التقنيه المهجنه أيون كروماتوغرافي– مطيافيه بلازما الحث المقترن. ان ميكانيكيه فصل المعقد Ce(IV)-DFOM التي تم الحصول عليها من خلال تغير الاس الهيدروجيني وتركيز البفر في الطور المتحرك أظهرت ان الفصل يستند على تبادل كأتيوني. على الرغم من عدد الدراسات في الادبيات التي تؤكد ان نوعيه هذه المبادلات تستخدم عادتاً لفصل المركبات الانيونيه, أثبتت قدرتها على فصل المركبات الكاتيونيه. العمود الذي تم تحضيره أظهر أختلاف بالمقارنه مع أعمده تجارية.