POLIMERY

Application of sulfonate polymer for detection of cations in industrial wastewater by capillary liquid chromatography

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Abstract: Poly(3-sulfopropylmethacrylate) was obtained and used for the separation of monovalent inorganic cations (Na^+, NH_4^+ and K^+) in industrial ion exchange chromatography of wastewater. The influence of monomer, porogen and polymerization time on the performance of the capillary column was investigated. The cation exchange capacity (CEC) and back pressure in high flow rate systems were determined. Optimal column preparation conditions were obtained using 40% monomer (30% SP : 10% EDMA), 60% porogen, 60°C temperature and 24-hour polymerization time.

Keywords: chromatography, ion exchange, methacrylate, monovalent cations, monolithic column.

Zastosowanie polimeru sulfonianowego do wykrywania kationów w ściekach przemysłowych metodą kapilarnej chromatografii cieczowej

Streszczenie: Otrzymano poli(3-sulfopropylometakrylan), który zastosowano do rozdziału jednowartościowych kationów nieorganicznych (Na^+, NH_4^+ i K^+) w przemysłowej chromatografii jonowymiennej ścieków. Zbadano wpływ monomeru, porogenu i czasu polimeryzacji na wydajność kolumny kapilarnej. Określono pojemność wymiany kationów (CEC) i ciśnienie wsteczne w systemach o dużym natężeniu przepływu. Optymalne warunki przygotowania kolumny uzyskano, stosując 40% monomeru (30% SP : 10% EDMA), 60% porogenu, temperaturę 60°C i 24-godzinny czas polimeryzacji.

Słowa kluczowe: chromatografia, wymiana jonowa, metakrylan, kationy jednowartościowe, kolumna monolityczna.

The analytical instruments that separate molecules of biological importance play a crucial role in various industries, particularly pharmaceuticals, chemical engineering, and related fields. Liquid chromatography instruments are commonly used to separate samples constituents and or to extract and refine a wide range of natural and synthetic chemicals. Molecular separation technology is dominated by gel electrophoresis, capillary

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electrophoresis, and liquid chromatography. Column chromatography can be used to separate proteins, but due to the need for a complex stationary phase and the low resolution of such columns, its use can be difficult.

Monolithic columns are an alternative for separating proteins through a combination of mass permeability [1]. The advantages of using monolithic columns are easy preparation, the ability to be used in flexible conditions between monomers and cross-linkers, low reagent consumption, require a small number of samples and good stability. In addition, monolithic columns are part of organic polymers with relatively large pores [2].

Monolithic columns are divided into two categories: organic polymers and silica-based monoliths [3]. Monolithic columns are based on organic polymers such as polystyrene, polymethacrylate and polyacrylamide. Organic polymer-based monoliths have many advantages, including good pH stability, simpler production process, fast separation with good resolution, the system has flow pores, good mass transfer and permeability. However, the mechanical stability of monolithic columns based on organic polymers results in short service life and undesirable retention repeatability. In contrast, silica-based monolithic columns have high mechanical strength, but surface functionalization takes a long time.

Monolithic columns are attracting increased attention and evolving in manufacturing and application. Polymer monolithic columns can be prepared using a combination of polymerization mixtures because the monomers and cross-linkers are inseparable components. Cross-linking and porogen processes facilitate the formation of porosity in monolithic stationary phase. Currently, polymer blend combinations are used to manufacture monolithic polymer synthesis. The organic polymer prepared using simple and single thermal preparation has an attraction and potential for environmental application [4]. The composition of compounds used in the polymerization process can influence the yield characteristics of monoliths, including polymethacrylate and polyacrylate monoliths [5, 6], as well as the separation process, particularly for macromolecules like nucleic acids, proteins, and peptides [7–10]. Post-modification, monolith-based organic polymers can interact with organic ions and make slight analytical separations, where ion conversion can occur. However, to maximize ion exchange in monolith columns, modification of inorganic anions and cations is necessary [11–14]. In ion chromatography, the ion-splitting process occurs via electrostatic contact between the stationary phases.

The sulfonate silica hybrid polymer column with a strong cation exchange site was potentially applied to detect and separate macromolecules, peptides, and proteins in capillary electrochromatography [15–17]. The separation technique using a sulfonate silica hybrid polymer can overcome the challenges of proteomic and glycomic analytics [12]. It was fabricated using a straightforward and efficient one-pot fabrication technique [16, 17].

However, the combination using silane monomer was still used.

In capillary chromatography, sulfonate methacrylate-monolithic columns can be fabricated using various compounds, including anilines, alkylbenzenes, and phenols. Because sulfonate groups are widely used in capillary chromatography, a simple and environmentally favorable approach for producing sulfonate monolithic polymer is required.

This study aims to prepare a polymer monolithic column with sulfonate groups using a one-pot approach to reduce time consumption during post-modification and find a simple test preparation. The cation exchange site involved directly from 3-sulfopropyl methacrylate (SP) as a functional monomer to introduce the sulfonate functional group. The performance of sulfonate polymer will be observed based on the percentage and portion of functional monomer and polymerization conditions. Then, chromatographic performance was determined through the detection of inorganic cations.

EXPERIMENTAL PART

Materials

3-Sulfopropylmethacrylate (SP) and ethylene dimethacrylate (EDMA) (97%) were supplied from Wako (Tokyo, Japan). 2,2-Azobisisobutyronitrile (AIBN) and 1-decanol, 3-(Methacryloyloxy)propyltrimethoxysilane (3-MAPS, 98%), copper(II) sulfate, and butane-1,4-diol were purchased from Tokyo Chemical Industry (Tokyo, Japan). Advantec GS-590 water distillation system (Tokyo, Japan) was used to obtain pure water. Cation solutions were obtained from Nacalai Tesque (Kyoto, Japan). The reagent used to obtain the monolithic polymer column was chemically pure, and the cation solution was of HPLC grade.

Apparatus

The capillary liquid chromatograph was constructed from an L.TEX-8301 microfeeder with an L.TEX 8150 pressure sensor (L.TEX Corporation, Tokyo, Japan). A gas-tight MS-GAN 050 syringe (0.5 mL, Ito, Fuji, Japan) with a model 7520 injector (Rheodyne, Cotati, California, USA) was used as the pump. The injection volume was 0.2 μL, on a 100 × 0.32 mm i.d. microcolumn. A UV/VIS detector (UV-1575, JASCO, Tokyo, Japan) was used to detect cations. Data were acquired using a Chromatopac C-R7A (Shimadzu, Kyoto, Japan).

Preparation of sulfonate polymer column

A silica capillary tube was obtained from GL Sciences (Tokyo, Japan) dimensions of 0.32 × 0.45 mm. The first step was to clean the capillary tube using three separate solutions: 1M NaOH, deionized water, and 1M HCl at the end. The second step was to prepare the surface
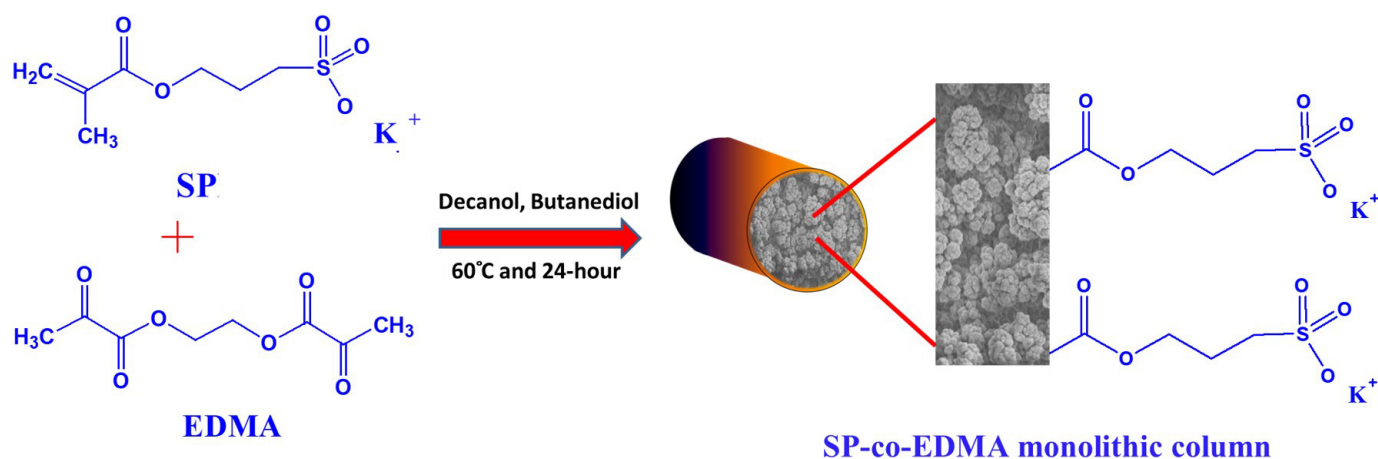
Fig. 1. Scheme of the expected reaction for the preparation of sulfonate polymer column

of the inner wall of the capillary tube using a solution of 30% (v/v) of γ-MAPS in acetone, additionally, providing the methacrylate groups. Capillary tubes were heat treated at 60°C for 24 h in water bath. The third step was drying the capillary tubes using nitrogen gas for 30 minutes. The polymer initiator solution consisting of monomer, crosslinker and porogen was prepared. 2 mg of AIBN was added to 0.1 mL of polymer solution and then mixed. Furthermore, the solution placed in ultrasonic vibrations for 5 min before being inserted into the capillary tube. The thermal polymerization conditions were carried out at 60°C for 24 h as initial thermal conditions, with the scheme of expected reaction shown in Figure 1. The last step was rinsing the capillary using a methanol to eliminate any leftover reagents and porogen solvents. An optimization method was conducted to prepare the sulfonate polymer column by evaluating various parameters that impact the polymerization process consisting of functional monomers (10, 20, 30 and 40%), percentage of porogen (60, 70, 80 and 90%), polymerization temperature (50, 60, 70 and 80°C) and polymerization time (6, 12, 18, 24 and 35 hours). Then, the number of the cation exchange capacity (CEC), back pressure system, and height value equivalent to a theoretical plate (HETP) of the column was investigated to determine the capability of the sulfonate polymer column.

RESULTS AND DISCUSSION

Effect of monomer on the CEC

In this work, a one-pot synthesis was developed on a sulfonate polymer column consisting of a cation exchanger for the separation process. The sulfonated polymer was prepared using SP as a functional monomer and ethylene dimethacrylate (EDMA) as a crosslinker. The sulfonate group was directly introduced into the polymer matrix from SP monomer as a functional monomer. The monomer to porogen ratio was studied by maintaining the crosslinker monomer ratio of 3:1 as shown in Table 1.

T a b l e 1. Composition of the sulfonate polymer column mixture

Column	Monomer % (v/v)	Cross-linker % (v/v)	Porogen % (v/v)
SP 1	7.5	2.5	90
SP 2	15.0	5.0	80
SP 3	22.5	7.5	70
SP 4	30.0	10	60

The percentage of SP could affect the performance of CEC, as shown in Figure 2. It was seen, that increasing the portion of SP as monomers affected the increase of CEC. The CEC could be characterized as the portion of sulfonate groups introduced by functional monomers. The polymerization conditions of the monolith column were maintained at 60°C for 24h as the initial condition. These polymerization conditions can affect the parameters of the monolith column. The CEC affected by increasing the percentage of monomers were 0.50, 0.52, 0.58, and 0.69 mequiv/mL, respectively. Furthermore, the standard deviation (σ) value is 0.85. The results indicate the capability of the sulfonate groups influenced the increase in monomer percentage. The cation exchange capacity increases linearly with the increase in the monomer. The
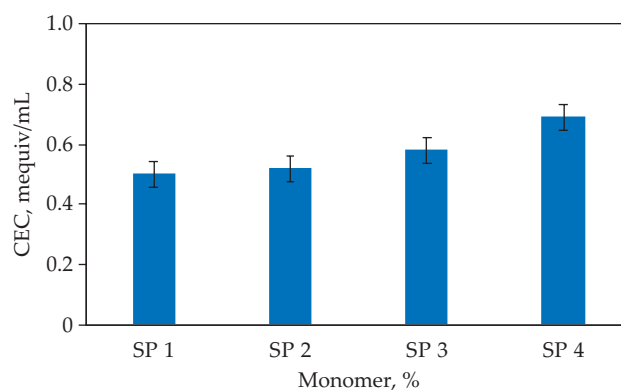

Fig. 2. Effect of monomer on the cation exchange capacity in sulfonate polymer column
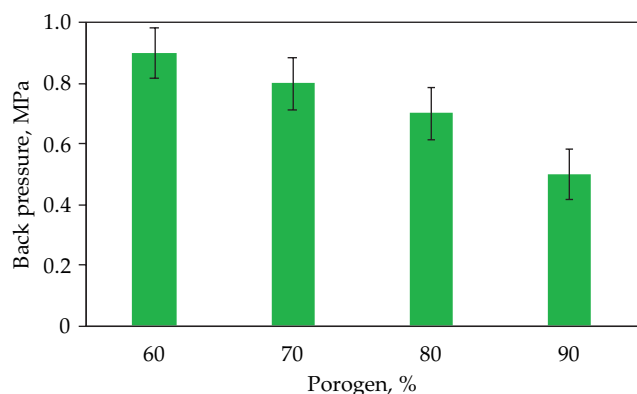
Fig. 3. Effect of porogen on back pressure at a flow rate of 4 μL/min using 10 mM CuSO$_4$ as eluent

Fig. 4. Effect of polymerization temperature on the CEC in sulfonate polymer column

SP monomer could play a massive role in the polymerization and sulfonation. The increasing number of functional monomers will affect the mechanical strength needed to determine stability in the CEC experiment [2]. In this study, 40% of monomer (30% of SP: 10% of EDMA) in the polymerization solution showed the optimal condition and affected the CEC.

Effect of porogen on back pressure

Figure 3 shows that increasing the amount of porogen from 60 to 90% during the polymerization reduced the back pressure in the system from 0.9 MPa to 0.5 MPa, with the standard deviation (σ) of 0.17. It shows a linearly proportional back pressure achieved of less than 0.9 MPa. The back pressure was dependent on the flow rate. The poly(ethylene glycol) methacrylate-based polymer column achieved 15 MPa while operating under 0.254 μL/min of flowrate using water as eluent [17]. Furthermore, increasing the amount of porogen in the polymerization can cause a decrease in system pressure. These indicate that the sulfonate polymer column potentially operates under a high flow rate. The low back pressure permitted the use of very high flow velocities, significantly improving separation [18]. The control monolithic stationary phase exhibited lower permeability and porosity values and higher column back-pressure, which could reduce the monolith pore structure in the control column, increasing the surface density of the stationary phase [19].

The effect of polymerization temperature on the CEC

Temperature conditions are an essential factor in the polymerization process, due to its importance in the formation of pores. The temperature of the porogen can be used to predict the exothermic heat released [5]. The degree of exotherm and heterogeneity is directly proportional to the percentage of monomer. The preparation temperature optimization is necessary. Polymer columns do not form properly at low temperatures. When polymerization was carried out at a temperature lower
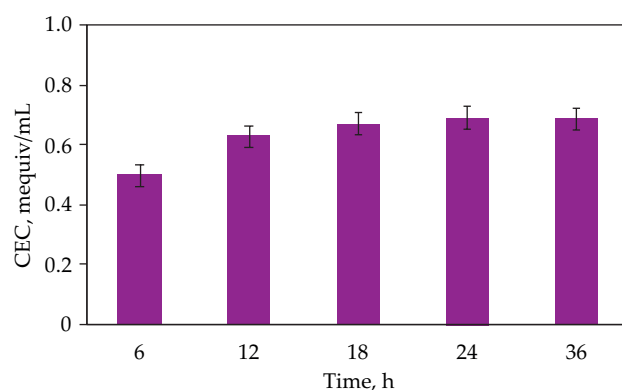
than 50°C, incorrect anchoring of the silanol group to the inner surface of the capillary and its release from the capillary were observed.

Meanwhile, high temperatures can cause homogeneity of the monolith, which can hold the inner walls of the capillaries. Therefore, polymers formed at high temperatures can increase system pressure. The polymerization process at 50°C reduced the pressure to 0.3 MPa. In contrast, polymerization at 80°C can increase the pressure by 1.2 MPa. Figure 4 shows the effect of temperature polymerization on the CEC in the sulfonate polymer column. Variety polymerization temperature affects the slightly significantly increasing number of CEC in 50, 60, 70 and 80°C were achieved at 0.66, 0.70, 0.71 and 0.72 mequiv/mL, respectively. Furthermore, the standard deviation (σ) was 0.03. Temperature plays a crucial role in determining polymer weight. Temperature variations affected molecular weight values, especially at higher molecular weights. Therefore, controlling temperature and reaction time is essential for achieving the desired molecular weight and properties in polymer synthesis [20].

Effect of polymerization time on the CEC

Long polymerization times can increase the cation exchange capacity. Polymerization was carried out at 6, 12, 18, 24 and 36 hours, increasing the CEC 0.50, 0.63, 0.67,
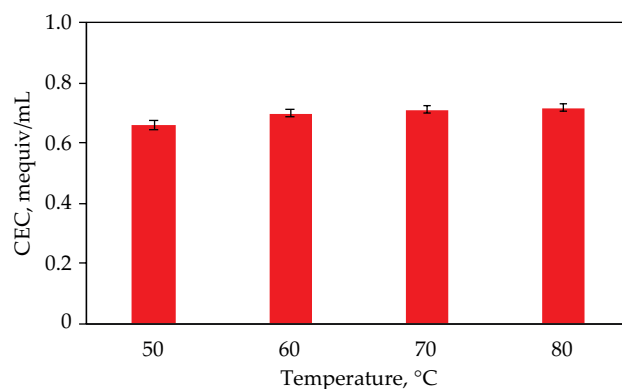

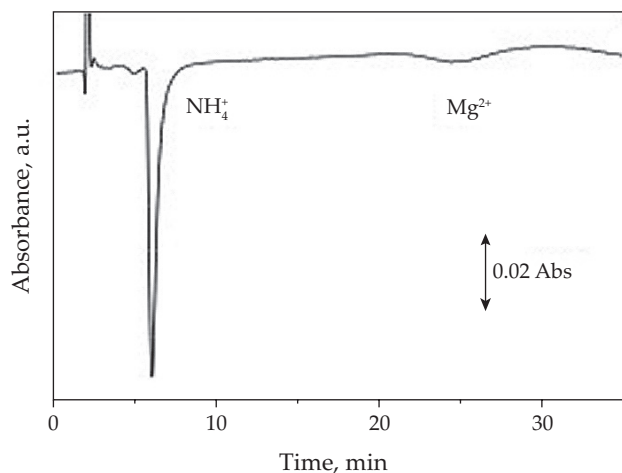Fig. 5. Effect of polymerization time on the CEC

Fig. 6. Separation of cations using sulfonate polymer column (100 mm, 0.32 mm i.d. × 0.45 mm o.d.); column (100 × 0.32 mm i.d.), eluent 5 mM of copper(II) sulfate, flow rate 4 μL/min, detection UV wavelength 210 nm and injection volume 0.2 μL

Fig. 7. Separation of monovalent cations using sulfonate polymer column (100 mm, 0.32 mm i.d. × 0.45 mm o.d.), column (100 × 0.32 mm i.d.), eluent 5 mM of copper(II) sulfate, flow rate 4 μL/min, detection UV wavelength 210 nm and injection volume 0.2 μL

0.69 and 0.69 mequiv/mL, respectively (Fig. 5). Furthermore, the standard deviation (σ) was 0.08. The research results indicated that the polymerization time was proportional to the CEC. The polymerization time significantly affects the pore size distribution, total porosity, and morphology [21] continuous poly[(1,2-bis(p-vinylphenyl)].

Detection of inorganic cations using ion-exchange mode capillary liquid chromatography

Separation of cations on sulfonate polymer column

The electrostatic interaction occurred between negatively charged particles as cations exchanger sites in columns. Cation exch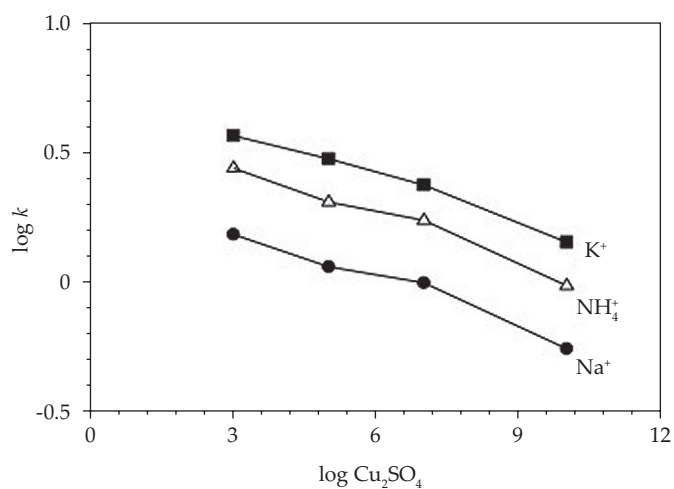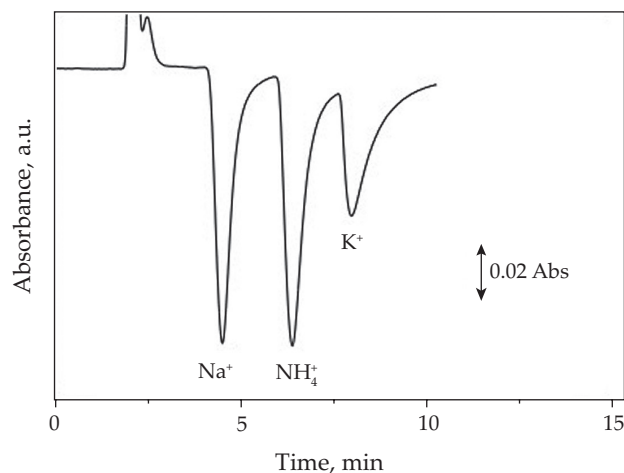ange occurs between the sulfonate groups interacting with the positively charged compound. The sulfonate group was obtained from the SP monomer and contributed as a cation exchanger site in the stationary phase. Sulfonate polymer columns have a variety of cation exchange characteristics. However, it is necessary to examine the cation characteristics using inorganic cations. Direct UV absorption at 210 nm was applied to detect cations on a column, and copper (II) sulfate was used as the eluent. The ability of the sulfonate column could be evaluated by separating monovalent cations and divalent cations. In addition, monovalent cations and divalent cations could be measured in the efficiency and specificity of the cation exchange column. Monovalent and divalent cations work through a mixture of NH_4^+ and Mg^{2+}, which are injected as analytes, as shown in Fig. 6 and Fig. 7.

Fig. 8. Effect of retention of inorganic monovalent cations (Na^+, NH_4^+ and K^+). Column: sulfonate polymer column (100 mm, 0.32 mm i.d. × 0.45 mm o.d.), column (100 × 0.32 mm i.d.), eluent 5 mM of copper(II) sulfate, flow rate 4 μL/min, detection UV wavelength 210 nm and injection volume 0.2 μL
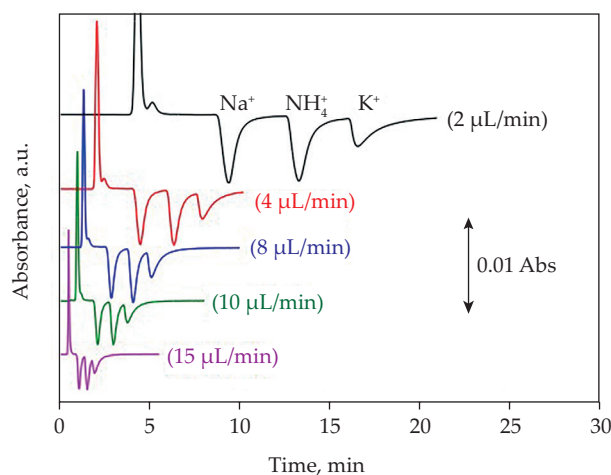

Fig. 9. Effect of separation at different flow rates. Column: sulfonate polymer column (100 mm, 0.32 mm i.d. × 0.45 mm o.d.), column (100 × 0.32 mm i.d.), eluent 5 mM of copper(II) sulfate, detection UV wavelength 210 nm, injection volume 0.2 μL and flow rate 2, 4, 8, 10 and 15 μL/min
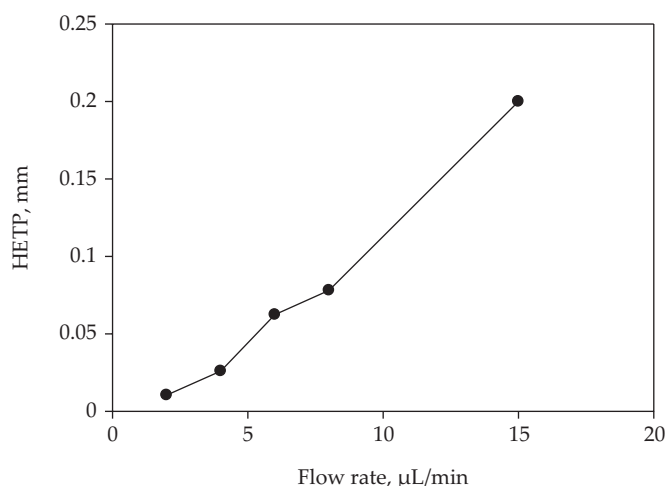
Fig. 10. Effect of flow rate on HETP

The operating conditions (Figs. 6 and 7) included 1 mM each of NH_4^+ and Mg^{2+} column (100 × 0.32 mm i.d.), 5 mM of copper (II) sulfate as eluent, 4 µL/min for flow rate, UV detection 210 nm and injection volume of 0.2 µL. The experiments were carried out between positively charged cations and 5 mM of copper (II) sulfate from the eluent. Fig. 4 shows the separation performance of monovalent and divalent cations perfectly separated. Nevertheless, the expanded peak of Mg^{2+} was discovered. The sulfonate groups attached could retain the divalent cation in terms of Mg^{2+} for longer than NH_4^+. Furthermore, the radius ionic size of Mg^{2+} (0.079 nm) is smaller than NH_4^+ (0.140 nm) [22], which affects the elution process. Fig. 6 demonstrates the separation profile of monovalent cations (Na^+, NH_4^+ and K^+). It shows that the separation profile could be eluted on 100 mm of sulfonate polymer column, with the theoretical number of plates of Na^+, NH_4^+, and K^+ being 473, 1628 and 3812 plates/m.

Furthermore, the impact of copper(II) sulfate content was examined. Fig. 8 shows the logarithm of the retention factor (log k) for cations, which can be expressed as a logarithmic function of the eluent concentrations. Decreasing eluent concentration was observed to result in longer retention time for each cation. The separation process of the cations on the sulfonate polymer column involved cation exchange, with copper ions acting as the carrier ions. The elution of Na^+, NH_4^+ and K^+ based on the variety of eluent concentrations occurred with excellent stability. The elutions were validated by calculating the correlation coefficients of elution using several concentrations of eluents. The plot of each cation has a linear relationship, as indicated by the correlation coefficient (R^2) of Na^+, NH_4^+ and K^+ were 0.9701, 0.9773 and 0.9845, respectively, proving the excellent linearity of the present method. These indicated that this elution of eluent satisfied performed and stable under various eluent concentrations.

Mechanical stability of sulfonate polymer column for separation of monovalent cations

The sulfonate polymer column has several advantages, including its robust mechanical strength and low flow resistance, enabling efficient operation at a high flow rate and delivering rapid separation rates. Flow rate variations in this study consisted of (2, 4, 6, 8, and 15 µL/min). the samples used were Na^+, NH_4^+ and K^+ with a size of 1mM each. The effect of flow rate on the separation of Na^+, NH_4^+ and K^+ in this study is shown in Fig. 9. The high flow rate is due to the good mechanical strength and high flow resistance when separation occurs in the monolith column. Flow rate variations in this study consisted of (2, 4, 6, 8, and 15 µL/min). the samples used were Na^+, NH_4^+ and K^+ with a size of 1mM each. The effect of flow rate on the separation of Na^+, NH_4^+ and K^+ in this study is shown in Fig. 9. From the chromatogram obtained, the decrease in flow rate was proportional to the decrease in elution time. Other conditions are affected by the theoretical plate equivalence height. HETP estimated the efficiency of the column packed with a duet stationary phase [23]. Figure 9 shows the effect of plate equivalence on the mobile phase flow rate. HETP always increases when the mobile phase flow rate increases. Based on Fig. 10, the HETP had a tenfold rise when the flow rate was adjusted from 2 to 15 µL/min. The 15 mL/min flow rate can be optimal for great column efficiency in these conditions.

CONCLUSIONS

A sulfonate polymer column containing strong cation exchange groups derived from 3-sulfopropyl methacrylate, which introduced sulfonate groups, was successfully prepared. A sulfonate polymer column was prepared to detect monovalent cations (Na^+, NH_4^+ and K^+). The sulfonate functional group was incorporated into a one-step heat treatment process, which included the polymerization of the sulfonate polymer column, which was influenced by the percentage of monomer, porogen and polymerization conditions in terms of cation exchange capacity (CEC) and back pressure. The optimal conditions for preparing the sulfonate polymer column were obtained: 40% SP, 60% porogen, 24 h polymerization time and 60°C polymerization temperature. Furthermore, the analysis showed that the monovalent cation was identified using UV detection at a wavelength of 210 nm with copper(II) sulfate as the eluent. Sulfonate polymer columns have the potential to perform high flow rate operations.

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