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Article

Per-Aqueous Liquid Chromatography for the Separation of Polar Compounds-Preparation and Characterization of Poly(ethylene oxide-*co*-dimethylsiloxane) Based Stationary Phase

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Poly(ethylene oxide-*co*-dimethylsiloxane) was thermally immobilized on silica particles-Si(PEO)-to separate small polar compounds with water-rich mobile phases. Poly(ethylene oxide-*co*-dimethylsiloxane) content on Si(PEO) stationary phase was optimized using a central composite design. Infrared spectroscopy, scanning electron microscopy, and thermogravimetric analysis morphologically and structurally characterized the optimized material. Separation of standard test mixtures showed that the Si(PEO) phase had a typical reversed-phase elution order. However, the Si(PEO) phase retained polar compounds better than C_{18} or aqueous C_{18} phases under water-rich mobile phases. Under this condition, small changes in the acetonitrile fraction resulted in a marked increase in the retention of some polar drugs on the Si(PEO) phase. A typical condition observed in *per* aqueous liquid chromatography separations, a more environmentally friendly liquid chromatography approach. On the other hand, hydrophobic compounds showed lower mass transfer rates due to their low solubility in the aqueous mobile phase. Thus, the Si(PEO) phase was more suitable and efficient for separating polar or hydrophilic compounds.

Keywords: HPLC, stationary phase, poly(ethylene oxide-*co*-dimethylsiloxane), polar compounds, aqueous mobile phase

Introduction

High-performance liquid chromatography (HPLC) is the most comprehensive analytical technique for organic compound analysis, being applied in the most diverse branches of the industry. More than 85% of these applications occur in the reversed-phase, RP-LC mode, employing mobile phases composed of water and an organic solvent, methanol or acetonitrile, and silica-based C_{18} stationary phases.[1] Despite the fast and efficient separations in RP-LC for the most diverse applications, hydrophilic and polar compounds present low retention and poor separations in this mode.[2,3] A successful alternative for analyzing these compounds was the advent of hydrophilic interaction liquid chromatography (HILIC).[3-6] HILIC is characterized by a hydrophilic stationary phase, typical of normal-phase liquid chromatography (NP-LC), and a mobile phase containing a high content of acetonitrile (70-95%).[4,5,7,8] The separation mechanism is based on the distribution of polar compounds between the dense organic layer in the mobile phase and a thin water-rich layer on the surface of the stationary phase, ensuring adequate retention and efficient resolution of the compounds.[9] The number of publications in the last two decades using the HILIC mode demonstrates its efficiency in elution and separation of polar compounds.

However, the acetonitrile amount consumed in the analyzes and the organic waste generated in HILIC mode cannot be neglected.[10,11] It is estimated that one HPLC equipment, regularly in operation, generates about 1.5 L *per* day or approximately 500 L *per* year of organic waste.[12,13] Considering the number of HPLC equipment operating in pharmaceutical companies, quality control laboratories, academic institutions, etc., around the world, millions of liters *per* year of organic, toxic, and volatile waste are generated when HILIC mode is used.

Given this fact, greener chromatographic separations, especially of polar compounds, in liquid chromatography has been the target of growing interest in recent years. Green liquid chromatography focuses on reducing the consumption of organic solvents, either by reducing the

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dimensions of both the chromatographic column and/or the diameter of the stationary phase particles either by replacing methanol/acetonitrile with less toxic solvents such as ethanol, propylene carbonate, or superheated water.[12-17] In these situations, there is a need for instrumental adaptation or specific instrumentation, like ultra-high-efficiency liquid chromatography (UHPLC), nano-LC (nano liquid chromatography), etc., due to the increase of system backpressure or the increase of mobile phase viscosity.[12,15,18-20] This makes the analysis process even more expensive and impracticable for most laboratories.

An efficient alternative to promote green liquid chromatography in the separation of polar compounds has been *per*-aqueous liquid chromatography (PALC).[10,21-26] In PALC mode, polar compounds are separated by water-rich mobile phases (70-100%) using more hydrophilic stationary phases.[10] Analyses employing this approach have been economical since they use conventional HPLC equipment and significantly reduce waste disposal costs. The separation in PALC mode is driven by different mechanisms with contributions from hydrophobic, hydrophilic, ion-pair, and ion-exchange interactions, which distinguishes it from the other modes.[21,23]

Stationary phases for PALC mode are objects of study in the area and aim at selectivity in the polar compound separation.[25-29] Several materials have been proposed to separate polar compounds using water-rich mobile phases, with the same efficiency achieved by HILIC phases.[22,23,25-27,29] In our view, the development of these separation materials has required a significant number of steps, using specific, high purity, and high-cost reagents, making the preparation less repeatable and relatively expensive. Our proposal aims to produce a repeatable and low-cost stationary phase material by the thermal immobilization of a hydrophilic polymer on the silica particle surface. The main advantages of thermal immobilization of pre-synthesized polymers onto inorganic oxide particles are the strong fixation of thin polymeric layers on inorganic oxides, regardless of their surface reactivity; the fine adjustment of the material selectivity as a function of the polymer functional groups; the low cost of the polymer; and the simple preparation.[30-35] In this work, the thermally immobilized poly(ethylene oxide-*co*-dimethylsiloxane) (PEO) on silica particles for the polar compound separation using highly aqueous mobile phases was evaluated. Poly(ethylene oxide-*co*-dimethylsiloxane) has hydrophilicity characteristics, glass transition temperature below –60 °C, and positive properties for equilibrium systems, independent of adsorption mechanisms.[36,37] Furthermore, PEO and poly(ethylene glycol) (PEG) are pointed out as promising materials for separation processes using less toxic mobile phases[10,13,35] and to our knowledge, PEO has not been evaluated as a stationary phase for the HPLC columns. PEO was studied as a co-polymer with polydimethylsiloxane (PDMS). The PDMS block was chosen for the copolymer fixation on the silica support since polysiloxanes promote chemical bonds with silanol groups on the silica surface.[38,39] The stationary phase was characterized chromatographically for the retention mechanism using highly aqueous mobile phases. The PEO stationary phase was also evaluated for the separation of small polar compounds using water-rich mobile phases.

Experimental

Materials and methods

All compounds used for the chromatographic testing were analytical reagent-grade from Sigma-Aldrich (St. Louis, MO, USA) and not further purified. HPLC-grade methanol from JT Baker (Phillipsburg, NJ, USA) and ultrapurified water, Gehaka Master System MS3000 (São Paulo, Brazil) were used to prepare the mobile phases. Analytical reagent-grade chloroform, hexane, and toluene, all from Tedia (Rio de Janeiro, Brazil), were used without further purification. The mobile phases were prepared volumetrically from individually measured amounts of each solvent and filtered using 0.22 μm Nylon membrane filters from Millipore before use. Chromosorb silica (5 μm of particle size) from Varian (Palo Alto, CA, USA), having a mean pore size of 12.6 nm and about 350 $m^2\ g^{-1}$ specific surface area was used as the chromatographic support. Poly(ethylene oxide-*co*-dimethylsiloxane) (PEO) (chemical structure in Figure S1, Supplementary Information (SI) section), viscosity 130 Cst, average molar mass ca. 5700 g mol^{-1} (Figure S2, SI section), was purchased from UCT Specialties (Bristol, CT, USA). Potassium salts (KH_2PO_4 and K_2HPO_4) were obtained from Merck (Darmstadt, Germany). The pesticide standards 2,4-dichlorophenoxyacetic acid (2,4-D) (98%), methomyl (99%), thiophanate-methyl (98%), carboxin (98%), captan (98%), diuron (98%), terbuthylazine (98%), and chlorpyriphos (98%) were obtained from Riedel-de-Haen (Seelze, Germany); paracetamol (*N*-acetyl-4-aminophenol) (97%), caffeine (99%), and diclofenac sodium (98.5%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of poly(ethylene oxide-*co*-dimetylsiloxane) thermally immobilized on silica

Silica was previously activated at 140 °C for 12 h and added to a 10% (m/v) solution of PEO in hexane at a specific

mass of 0.5 g_{PEO}/g_{SiO2}. The mixture was slowly stirred for 3 h and, after that, the solvent was allowed to complete evaporate under fume hood at room temperature for 4-5 days. The dried material was submitted to the thermal treatment. The optimal conditions for PEO immobilization on the silica particles were evaluated by a fractional factorial design followed by a central composite design. The type of solvent, immobilization temperature and time, and PEO loadings and their studied levels are presented in Table 1. All experimental design data are in SI section.

Table 1. Variables and their levels for the thermal immobilization of PEO on silica particles

Variable		Low level (−)	High level (+)
1	solvent	hexane	chloroform
2	immobilization temperature / °C	100	150
3	immobilization time / h	8	16
4	PEO loadings / (% m/m)	25	50

PEO: poly(ethylene oxide-*co*-dimethylsiloxane).

The polymer loading, immobilization time, and temperature conditions shown in Table 1 were chosen based on previous works.[30,34,35] Solvents were defined based on two criteria: total solubility of PEO and production of a balanced suspension in which particles do not settle. Fifteen stationary phases combining the factor levels (Table 1) to define the optimal conditions for the PEO immobilization on silica were prepared. The polymer coating of silica particles and the column efficiency were the responses evaluated in this study. After each thermal immobilization, the non-immobilized PEO were extracted by sequential washing with hexane, methanol, and methanol:water (1:1) and discarding of supernatant after centrifugation at 2500 rpm for 2 min. Si(PEO) phases were dried at 70 ºC for 30 min prior to the column packing.

Column packing

Stainless steel type 316 columns (50 mm × 4.0 mm internal diameter (i.d.)) were slurry packed using 10% (m/v) of the Si(PEO) phases in chloroform. A constant packing pressure of 60 MPa was used with methanol as propulsion solvent using a Restek packing in a box kit (Bellefonte, PA, USA). Prior to the chromatographic analyzes all columns were conditioned for 1 h with mobile phase at 0.5 mL min^{-1} using mobile phase MeOH:water (50:50, v/v).

Physicochemical and chromatography characterization of Si(PEO) stationary phase

Morphological and physicochemical characterizations of the Si(PEO) phase were performed by Fourier-transform infrared spectrometry (FTIR), using a Cary 630 FTIR spectrometer (Agilent, Santa Clara, CA, USA) equipped with an attenuated total reflectance (ATR) accessory. The IR spectral range was evaluated from 600 to 4000 cm^{-1} using a resolution of 4 cm^{-1} and a scan rate of 32 scans min^{-1}. Si(PEO) phase samples were analyzed by elemental analysis on a Model CHN-2400 PerkinElmer Analyzer (Shelton, CT, USA). The Si(PEO) phases were heated from 25 to 600 °C at 10 °C min^{-1} in a N_2 atmosphere, using a Discovery TGA-55 thermogravimetric analyzer from TA Instruments (New Castle, DE, USA). The morphological evaluation of the Si(PEO) particles was performed in a TESCAN scanning electron microscope Vega3 model with an X-ray microprobe (Tokyo, Japan).

The performance of all Si(PEO) phases was chromatographically evaluated by the separation of a test mixture: uracil (0.5 mg L^{-1}), benzonitrile (10.0 mg L^{-1}), benzene (10.0 mg L^{-1}), toluene (10.0 mg L^{-1}), and naphthalene (10.0 mg L^{-1}). Column efficiency (N), retention factor (k), resolution (R_s), and asymmetry factor (A_{s10}) were the chromatographic parameters used to determine the optimal preparation conditions of the Si(PEO) stationary phases.

The retention properties of the Si(PEO) stationary phases were determined by the separation of Tanaka test mixtures.[40] Tanaka test consists of four test mixtures submitted to the separation by the stationary phase under predefined mobile phase conditions. Tanaka test mixture 1 (TM1): uracil (0.5 mg L^{-1}), butylbenzene (20.0 mg L^{-1}), amylbenzene (20.0 mg L^{-1}), *o*-terphenyl (20.0 mg L^{-1}) and triphenylene (20.0 mg L^{-1}). Mobile phase: MeOH:water (80:20, v/v); Tanaka test mixture 2 (TM2): uracil (0.5 mg L^{-1}), caffeine (10.0 mg L^{-1}), and phenol (10.0 mg L^{-1}). Mobile phase: MeOH:water (30:70, v/v); Tanaka test mixture 3 (TM3) and Tanaka test mixture 4 (TM4): uracil (0.5 mg L^{-1}), benzylamine (10.0 mg L^{-1}) and phenol (10.0 mg L^{-1}). Mobile phases: MeOH:20.0 mmol L^{-1} of phosphate buffer (30:70, v/v at pH 7.60) and (30:70, v/v at pH 2.70), respectively for TM3 and TM4. All separations were evaluated at a flow rate of 0.5 mL min^{-1} at 40 °C. For comparison, Tanaka test mixtures were analyzed on a conventional C_{18} column (Kromasil C_{18}®, 150 mm × 4.6 mm, 100 Å, 5 µm particle size) and a polar-embedded C_{18} column (Symmetry Shield RP18, 150 mm × 4.6 mm, 100 Å, 3.5 µm particle size).

Separation of polar compounds

The optimized Si(PEO) phase was evaluated by the separation of active compounds from a drug used in rheumatism treatment composed of *N*-(4-hydroxyphenyl) ethanamide (300 mg), carisoprodol (125 mg), caffeine (30 mg), and diclofenac sodium (50 mg). The best separation was performed with mobile phase ACN:5 mmol L^{-1} ammonium acetate (1:99, v/v), a flow rate of 0.5 mL min^{-1}, a temperature of 25 °C, and UV detection at 254 nm. Retention times in the chromatogram were confirmed by the individual analysis of standard solutions of each compound. The aqueous content and ammonium acetate concentration effects in the mobile phase on compound retentions were also evaluated.

The applicability of the Si(PEO) phase was also evaluated under the highly aqueous mobile phase by the separation of a pesticide mixture containing 2,4-dichlorophenoxyacetic (10.0 mg L^{-1}), captan (20.0 mg L^{-1}), carboxin (10.0 mg L^{-1}), chlorpyriphos (10.0 mg L^{-1}), diuron (5.0 mg L^{-1}), methomyl (10.0 mg L^{-1}), terbuthylazine (10.0 mg L^{-1}), and methyl thiophanate (5.0 mg L^{-1}). The pesticide mixture was also separated by a commercial column for comparison, Kromasil C_{18}®. The optimal chromatographic conditions for the separation of pesticide mixture were using mobile phases ACN:ammonium acetate 5 mmol L^{-1} solution (30:70, v/v), flow rate at 0.5 mL min^{-1}, oven temperature 25 °C, and UV detection at 254 nm.

All analyzes were performed by using an HPLC system (Waters Alliance e2695) (Milford, MA, USA) equipped with an autosampler, a quaternary gradient pump, and a photodiode array detector (Waters 2998 PDA). The software for control and data acquisition was Empower 3®.

Results and Discussion

Optimization of thermal immobilization of the Si(PEO) phases

Optimal conditions of immobilization temperature and polymer loading of PEO on silica particles were determined using a central composite design (CCD). The dispersion solvent and immobilization time were fixed as hexane and 16 h, respectively, after fractional factorial design study once higher percentages of PEO were obtained in these conditions (Table S1 and Figure S3, SI section). Triplicate at the central point and an axial factorial design were carried out for the CCD experiments, measuring the PEO immobilized on silica particles by elemental analysis. Carbon percentages for each experiment designed by CCD are presented in Table 2.

The results presented in Table 2 were statistically analyzed regarding of regression model for the thermal immobilization of PEO on silica particles. The reduced cubic model, equation 1, was the best fit for the experimental data of CCD. The model validation was evaluated by analysis of variance (ANOVA) (Table S2, SI section). The reduced cubic model was significant at the 95% confidence interval, with R^2 (coefficient of determination) and adjusted R^2 values of 97.73 and 92.44%, respectively. Figure 1 shows the three-dimensional response surface of carbon percent of the relationship between temperature immobilization (T) and PEO loadings (L). The three-dimensional response surface graphs were plotted using the Design-Expert® software trial.[41]

$$C(\%) = 6.82 + 0.34T + 0.51L - 0.38T^2 - 0.82L^2 + 0.66T^2L - 1.35TL^2 \quad (1)$$

Table 2. Carbon percentages of stationary phases prepared by the thermal immobilization of PEO on silica particles according to the central composite design experiments

Si(PEO) phase	Real value		Coded value		C / %
	Temperature / °C	PEO loading / (% m/m)	T[a]	L[b]	
1 (7)[c]	100	25	−1.0	−1.0	5.28
2 (4)	150	25	+1.0	−1.0	3.56
3 (8)	100	50	−1.0	+1.0	7.92
4 (1)	150	50	+1.0	+1.0	5.63
5 (2)	125	37.5	0	0	6.83
6 (9)	125	37.5	0	0	6.40
7 (5)	125	37.5	0	0	7.23
8 (10)	90	37.5	−1.4	0	5.59
9 (6)	160	37.5	+1.4	0	6.57
10 (11)	125	20	0	−1.4	4.49
11 (3)	125	55	0	+1.4	5.94

[a]T: coded values for the immobilization temperature; [b]L: coded values for PEO loadings; [c]in bracket: the order of experiment execution. $T = \frac{temp - 125}{25}$; $L = \frac{PEO - 37.5}{12.5}$. PEO: poly(ethylene oxide-*co*-dimethylsiloxane).

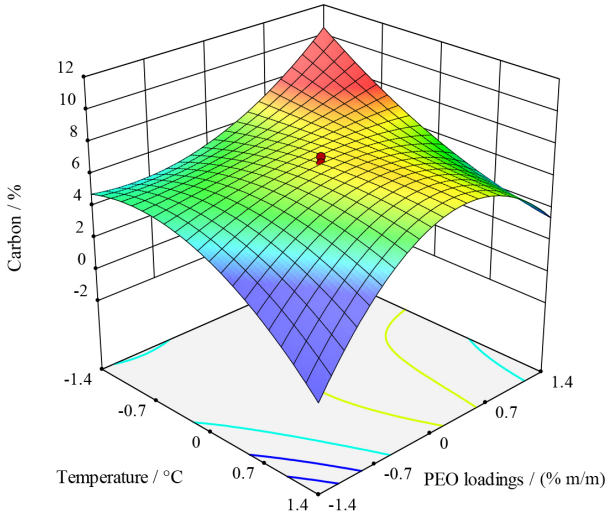

Figure 1. Response surface for carbon percentages of HPLC stationary phases prepared under different immobilization temperatures and PEO loadings.

A higher percent of carbon can provide a better chromatographic support coating minimizing undesirable interactions of polar solutes with active sites from the support surface. So, the optimization of temperature and polymer loading in the thermal immobilization of PEO aimed at the highest percent of carbon in the stationary phases. According to Figure 1, the optimal thermal immobilization of PEO is obtained in an empirical condition: lower temperatures and higher polymer loadings, which do not assure the required PEO fixation on the support surface. The highest percent of carbon for the stationary phases occurs when the coded values for polymer loading and immobilization temperature are in the ranges, respectively ca. 0.2-0.6 (40-45% m/m) and ca. 0.0-0.7 (125-140 °C). So, the best conditions for the thermal immobilization of PEO onto silica particles were chosen as 125 °C and 40% m/m for temperature and polymer loading, respectively.

Morphological and structural characterization of the Si(PEO) phases

The morphology of the stationary phase particles plays a fundamental role in the performance of HPLC columns. How the particles will be packed in stainless steel columns the more uniform the particles, the better the column will be packed and, consequently, the better the chromatographic performance. As can be seen in the micrographs in Figure 2, the Si(PEO) particles remain spherical and without agglomerations after the thermal immobilization. The Si(PEO) phase presented uniform particle sizes, as better seen in Figure 2a. Both particle spherical shape and size homogeneity are the adequate requirement for a compact and efficient column packing of Si(PEO) particles. The Si(PEO) phase particles possess a thin PEO layer, which is not noticeable in scanning electron microscopy (SEM) images due to their sub-nanometer scale thickness.[42] Also, the SEM micrographs indicated that the washing steps were efficient in removing non-immobilized material.

The Si(PEO) phase thermal stability was analyzed by thermogravimetric analysis. Figure S4 (SI section) presents the thermogravimetric (TG) curves for the pure PEO polymer and the optimized Si(PEO) phase. The Si(PEO) phase showed thermal stability up to about 100 °C when the loss of adsorbed water on the stationary phase silica support begins.[43] The most accentuated mass loss of Si(PEO) phase is registered from 140 °C due to the ethylene oxide copolymer decomposition, while after 260 °C occurs

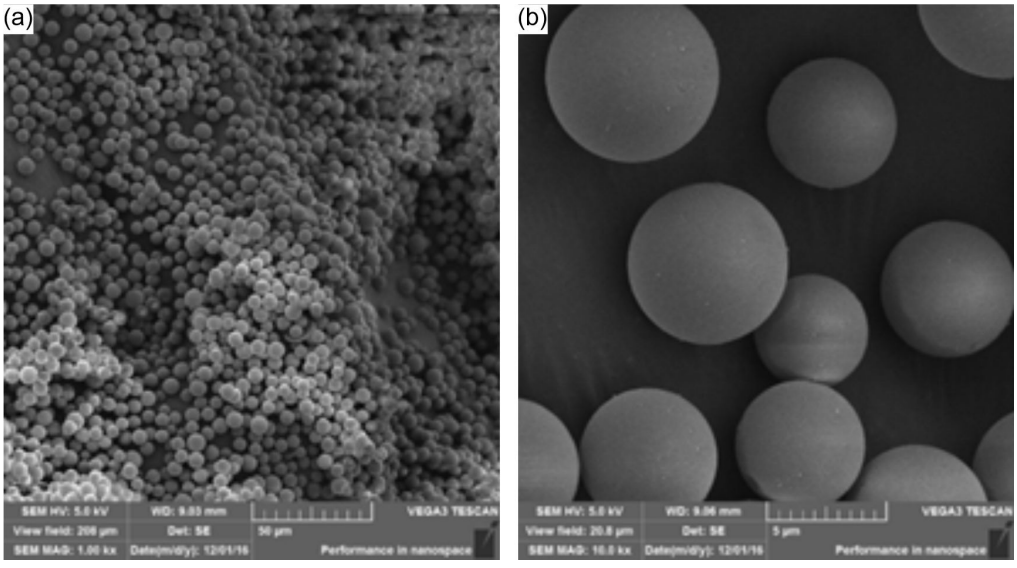

Figure 2. SEM images of Si(PEO) particles with (a) 1000× and (b) 10000× magnification.

the polydimethylsiloxane block decomposition.[44] The mass losses of PEO in the Si(PEO) phase occur 80 °C lower than those recorded in the pure polymer, Figure S4a. Despite this, the thermal immobilization of PEO on silica was below the depolymerization temperature.[45]

Infrared spectroscopy was used to evaluate the presence of ethylene oxide on Si(PEO) particles. The FTIR spectra of the optimized Si(PEO) phase, pure PEO and bare silica are shown in Figure 3. PEO polarity can be confirmed by the strong band in 3450 cm^{-1}, attributed to the O–H stretch arising from adsorbed water molecules and the –OH terminations of ethylene oxide in the monomeric units of PEO.[44,46] The PEO fingerprint region in the FTIR spectrum, Figure 3a, is identified by peaks 1271, 1341, and 1460 cm^{-1}, which are characteristics of CH_2 twisting, CH_2 wagging, and CH_2 scissoring, respectively.[46] These peaks were overlaid by an intense peak at 1100 cm^{-1}, resulting in a peak broadening seen in the FTIR spectrum of the Si(PEO) phase, Figure 3c. Moreover, low-intensity signals attributed to the C_{sp3}–H stretching are observed in the Si(PEO) phase spectrum at 2890 and 2960 cm^{-1}, indicating the presence of carbonaceous material in the inorganic oxide.

Retention mechanism of Si(PEO) phase using highly aqueous mobile phases

Chromatographic retention in the Si(PEO) phase was evaluated by separating Tanaka test mixtures. The Tanaka test is established worldwide as one of the most important tests to evaluate the HPLC column selectivity. The four Tanaka mixtures evaluate the hydrophobicity (k_{PB}), hydrophobic selectivity (α_{CH2}), steric selectivity ($\alpha_{T/O}$), hydrogen-bonding capacity ($\alpha_{C/P}$), total ion exchange capacity ($\alpha_{B/P\ pH\ 7.6}$), and acidic ion exchange capacity ($\alpha_{B/P\ pH\ 2.7}$). These parameters permit simplifying the column comparison. In general, the first three parameters measured obtained by the Tanaka test (α_{CH2}, $\alpha_{T/O}$, and $\alpha_{C/P}$) are related to the hydrophobic interactions of the stationary phase with the hydrophobic compounds of the mixtures, while the parameters $\alpha_{C/P}$, $\alpha_{B/P\ pH\ 7.6}$, $\alpha_{B/P\ pH\ 2.7}$ are related to the hydrophilic interactions with polar compounds (acidic or basic). These parameters (Table S3, SI section) were plotted in a radar plot (Figure 4) for the Si(PEO) phase and compared with two commercial C_{18} columns, a conventional and a polar-embedded group (also called

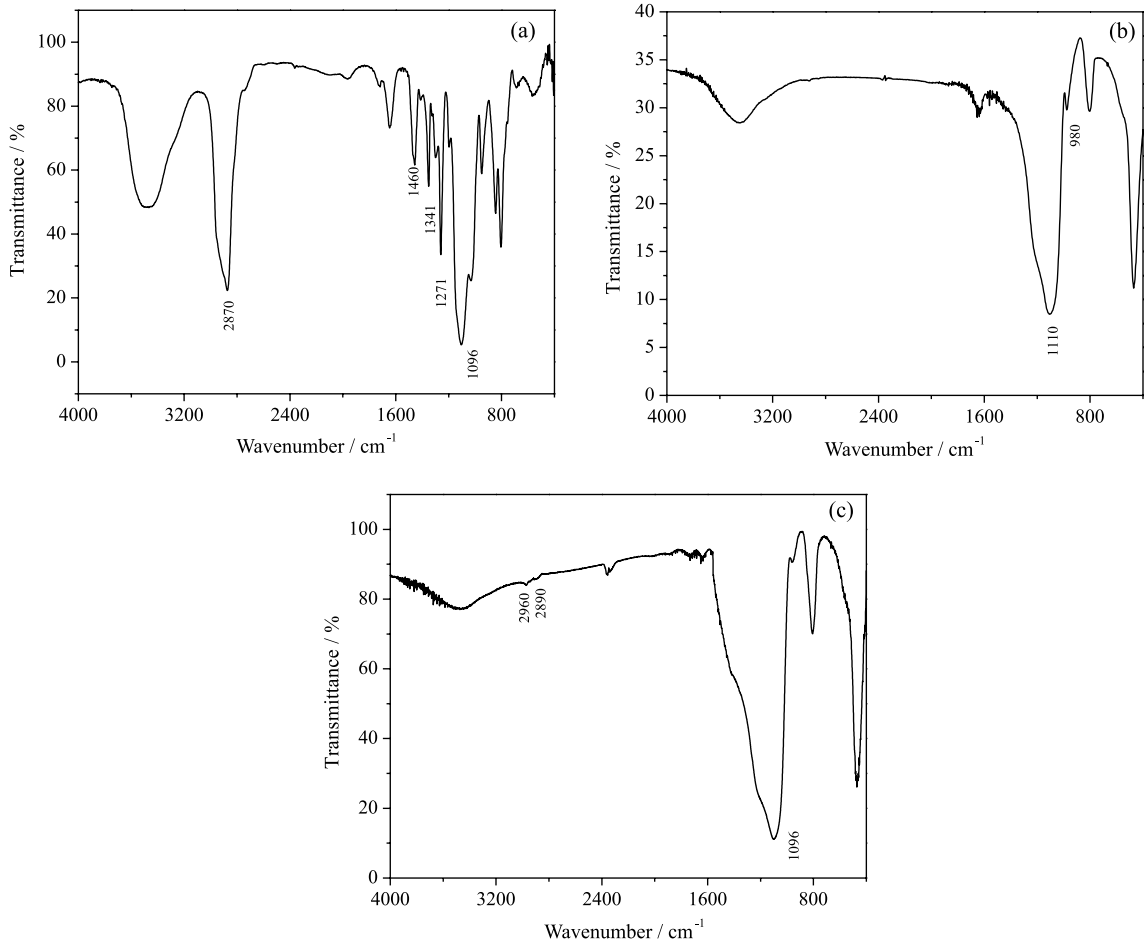

Figure 3. FTIR (ATR) spectra of (a) pure PEO, (b) bare silica, and (c) optimized Si(PEO) phase.

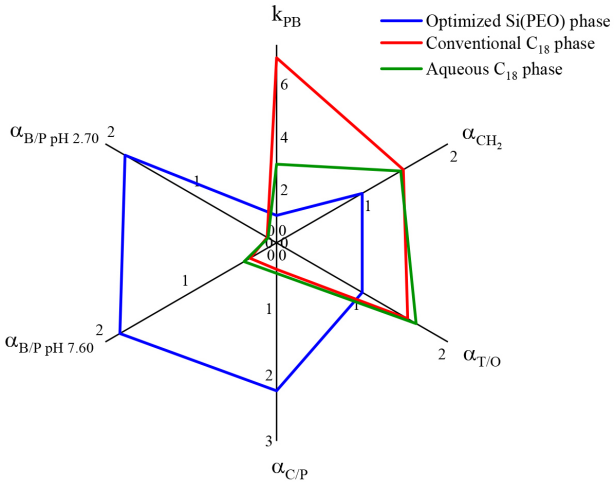

Figure 4. Radar plot for the retention parameters obtained by the separation of Tanaka test using the optimized Si(PEO), conventional C_{18}, and aqueous C_{18} (polar-embedded) phases.

the aqueous C_{18} stationary phase). The radar plot provides a visual representation of the global selectivity of each stationary phase in this virtual n-dimension space.[47,48]

The Si(PEO) phase separated the compounds of the first mixture of the Tanaka test (TM1) only when the proportion of methanol in the mobile phase was changed from 80 to 30% (Figure S5, SI section). Under this condition, the elution order of the compounds was consistent with the reversed-phase mode. Therefore, the Si(PEO) phase has low hydrophobicity compared to conventional reversed stationary phases, as seen on the k_{PB} axis of the radar plot, Figure 4. Moreover, the Si(PEO) phase has better selectivity for the polar compounds of the Tanaka mixtures, resulting in a larger area in the "hydrophilic region" of the radar plot. This region consists of the parameters associated with the hydrogen bonding capacity and ion exchange capacities. Benzylamine, the basic compound of the Tanaka mixtures, showed no peak broadening or tailing (A_{s10} = 1.05 and A_{s10} = 1.25) in the Si(PEO) phase (Figures S6a and S6b, respectively), suggesting that hydrophilic interactions with the PEO layer rather than residual sites on the chromatographic support occurred. Thus, the retention of polar compounds on the Si(PEO) phase may be associated with dipole-dipole or hydrogen bonding interactions with the ethylene oxide chains.[49] On the other hand, C_{18} phases showed better selectivity for hydrophobic compounds, resulting in larger areas in the "hydrophobic region" of the radar plot. Despite operating in the reversed-phase mode, the separation of the Tanaka test suggests different retention mechanisms of Si(PEO) and polar-embedded C_{18} phases.

The retention mechanism in the Si(PEO) phase was better evaluated separating a mixture of polar drugs under different mobile phase conditions. A tablet containing paracetamol, diclofenac, and caffeine was crushed and completely dissolved in methanol. The pharmaceutical mixture was separated by the Si(PEO) phase using a mobile phase with different concentrations of additives (ammonium acetate) and percentages of the aqueous component. The Si(PEO) phase completely separated the pharmaceutical compounds only when the composition of the mobile phase was more than 75% aqueous component. As the aqueous fraction increased from 75 to 90%, the Si(PEO) phase surface was saturated with acetonitrile and water, slightly increasing the retention of compounds (Figure 5a). Above 90% aqueous fraction, small changes in the acetonitrile fraction resulted in significant increases in the retention of the polar compounds. This result is in accordance with retention mechanism of polar compounds in PALC (*per*-aqueous liquid chromatography) mode.[21-25,50]

In PALC, mobile phase additives can have a substantial influence on the compound retention, on the selectivity, and

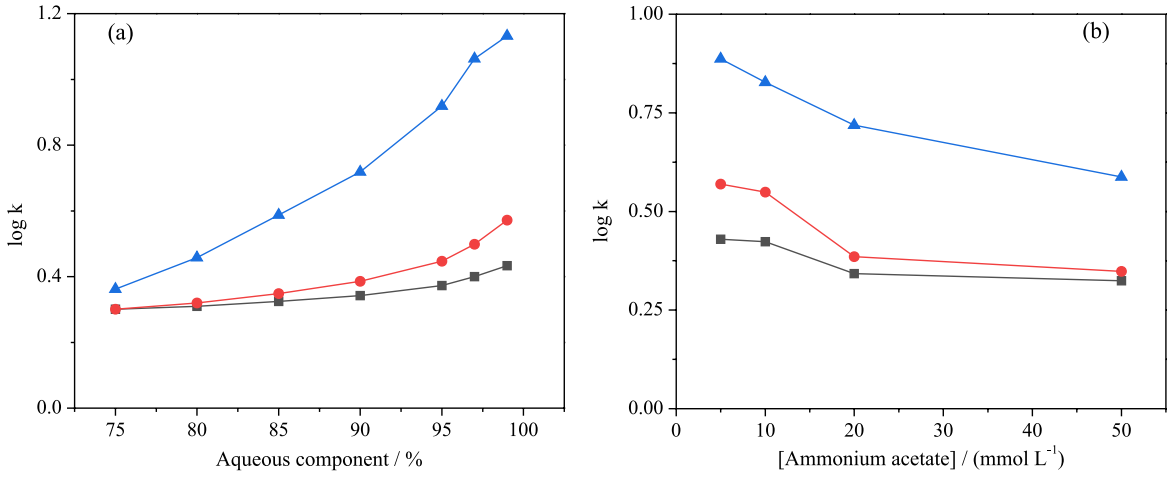

Figure 5. (a) Effect of mobile phase aqueous content and (b) additive concentration on compound retention using the optimized Si(PEO) phase. Chromatographic conditions: mobile phase, ACN:ammonium acetate. Flow rate 0.5 mL min^{-1}, temperature 25 °C, UV detection at 254 nm. (■) Paracetamol, (●) caffeine, (▲) diclofenac.

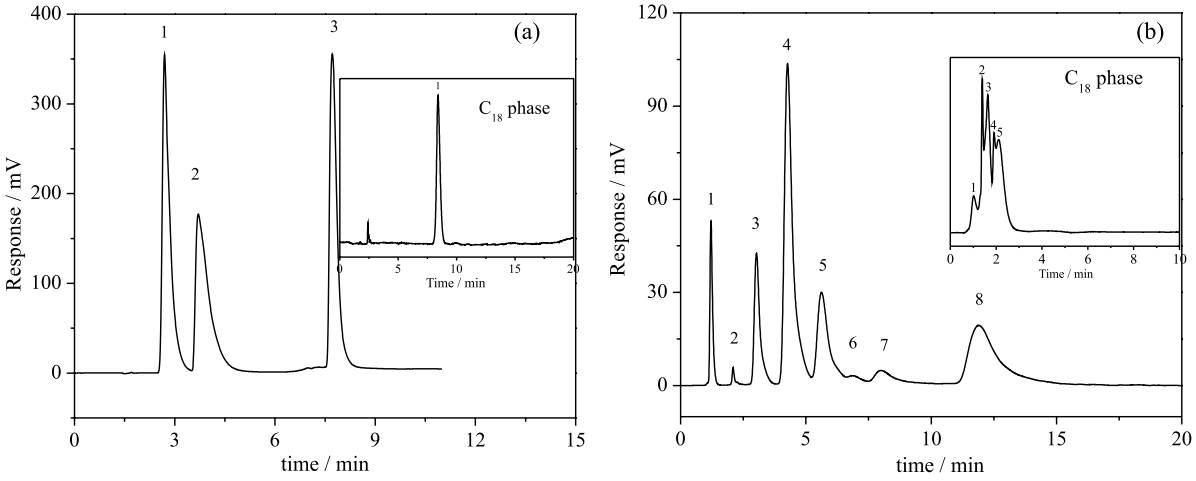

Figure 6. Separation of (a) drugs and (b) pesticides by the Si(PEO) and commercial C_{18} columns (inside featured). Compounds: (a) 1: paracetamol, 2: caffeine, 3: diclofenac sodium; (b) 1: 2,4-D; 2- methomyl; 3: thiophanate-methyl; 4: carboxin; 5: captan; 6: diuron; 7: terbuthylazine; 8: chlorpyriphos. Chromatographic conditions: (a) solvent A: sol. ammonium acetate 5 mmol L^{-1} and solvent B: acetonitrile. Gradient elution: 0-4 min (99% A); 4-5 min (99-80% A); 5-10 min (80% A); (b) ACN:ammonium acetate 5 mmol L^{-1} (30:70, v/v). Flow rate: 0.5 mL min^{-1}, oven temperature: 25 °C, UV detection: 254 nm.

even on the efficiency. So, the ionic strength of the mobile phase should be constant to prevent that residual sites contribute to the retention of polar compounds. Ammonium acetate was the aqueous component of the mobile phase for separations using the Si(PEO) phase. Figure 5b presents the effect of ammonium acetate concentration on the retention of pharmaceutical compounds.

According to Figure 5b, the retention time of pharmaceutical compounds slightly decreases with increasing the ammonium acetate concentration in the mobile phase. Higher salt concentrations increase the mobile phase elution force, reducing the retention time of polar solutes. Additionally, the salt ions suppress the electrostatic interactions among polar compounds and ethylene oxide on the Si(PEO) phase, causing loss of retention. This behavior is commonly observed for polar compounds with moderate retention factors in PALC stationary phases.[23,25,26]

The Si(PEO) phase separated the drug mixture with a mobile phase gradient starting with 99% of the aqueous component, Figure 6a. Under the same conditions, a typical reversed-phase did not separate the mixture due to the weak elution strength (inside featured in Figure 6a). Some interactions between compounds and ethylene oxide may have been responsible for chromatographic retentions. Moreover, gradient elution indicates effective PEO immobilization on the silica particles, with no loss of retention of the compounds observed during the analyzes. The column efficiency for the diclofenac sodium peak was 58,000 plates m^{-1} for this separation.

We investigated the potential of the Si(PEO) phase for separating a mixture of eight pesticides with different polarities. Separation was achieved by isocratic elution with 70% of the aqueous component in the mobile phase, as shown in Figure 6b. The polar pesticides in the mixture (2,4-D, methomyl, and thiophanate-methyl) eluted more efficiently and with more symmetrical peaks, while the more hydrophobic pesticides eluted as broad chromatographic peaks. These broadening peaks may be attributed to the slower mass transfer rate of the hydrophobic pesticides (terbuthylazine and chlorpyrifos) in the water-rich mobile phase. Gritti *et al.*[21] pointed out that this behavior is typical for stationary phases in PALC mode. The PALC mode is more efficient in separating compounds with moderate retention factors, such as moderately polar compounds.

The pesticide mixture was also analyzed using a commercial C_{18} column. The same mobile phase conditions were used for this, but no separation of the pesticides was observed (inside featured Figure 6b).

Conclusions

The results obtained for the stationary phase based on the thermal immobilization of poly(ethylene oxide-*co*-dimethylsiloxane) on silica particles indicated that this material has the potential to separate polar compounds as PALC stationary phase. Si(PEO) presented the ability to retain polar substances even in highly aqueous mobile phases. Some of the main attractions of the polymer-immobilized stationary phase are the fine-tune selectivity with the appropriate choice of the polymer and their simplicity and ease of preparation once the required materials and reagents are accessible and inexpensive. Although the order of compound elution in the Si(PEO) phase is in the reversed-phase mode, polar compounds

eluted with highly aqueous mobile phases, which is not the case with conventional reversed-phase columns. The PEO layer on the stationary phase promotes hydrophilic interactions with polar compounds, resulting in efficient chromatographic separations under the PALC mode. The use of highly aqueous mobile phases reduces waste and makes PALC an interesting alternative to the HILIC mode for the analysis of moderate polar compounds by liquid chromatography.

Supplementary Information

Supplementary information (additional characterization, statistical data, and chromatograms) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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