

# Tentacle-Type Zwitterionic Stationary Phase Prepared by Surface-Initiated Graft Polymerization of 3-[*N,N*-Dimethyl-*N*-(Methacryloyloxyethyl)ammonium] Propanesulfonate through Peroxide Groups Tethered on Porous Silica

Wen Jiang and Knut Irgum\*

Department of Analytical Chemistry, Umeå University, S-901 87 Umeå, Sweden

**A novel stationary phase with tentacle-type zwitterionic interaction layer was synthesized by free radical graft polymerization of 3-[*N,N*-dimethyl-*N*-(methacryloyloxyethyl)ammonium]propanesulfonate (SPE) from the surface of Kromasil porous silica particles. The polymerization was initiated by thermal cleavage of *tert*-butylperoxy groups covalently attached to the particle surface, and the material therefore carries a tentacle-type polymeric interaction layer with 3-sulfopropylbetaine functional moieties. The composition of the surface graft was determined by elemental analysis, and the surface charge was measured using photon correlation spectroscopy. The measured  $\zeta$ -potentials were close to 0 and nearly independent of pH, and the tentacle character of the interactive layers were evident from the lack of colloidal stability in the absence of salt (antipolyelectrolytic behavior) and a marked increase in column back-pressure when the concentration of perchloric acid or perchlorate salt was increased. The chromatographic properties were evaluated on columns packed with the functionalized material, and it was shown that this zwitterionic stationary phase could simultaneously and independently separate inorganic anions and cations using aqueous solutions of perchloric acid or perchlorate salts as eluents. The material was also capable of separating two acidic and three basic proteins in a single run, using gradient salt elution at constant pH.**

An ideal zwitterionic stationary phase is characterized by having ionic moieties containing both positive and negative charges in an exact balance. Consequently, although the surface density of charged moieties is high, the material has no net charge and is, thus, capable of accomplishing simultaneous separation of cations and anions. The zwitterionic functionality has attracted more interest in recent years, and many different zwitterionic stationary phases, including amino acids, “zwittergent” coated and covalently bonded types, have been developed on the basis of silica and polymeric materials, and their retention properties have been investigated. These stationary phases have been used to separate inorganic and organic anions and cations, as well as ampholytes, independently and simultaneously.<sup>1–6</sup>

In our recently published papers, we have described four different synthesis routes to produce covalently bonded zwitterionic stationary phases with variable charge balances.<sup>6</sup> These materials can accomplish the separation of inorganic anions and cations, independently and simultaneously, by using solutions of perchloric acid and perchlorate salt as mobile phases. It was found that minor differences in charge balance affect the separation profiles of inorganic ions, and although the separation properties of all these sorbents are mainly characteristic of zwitterionic materials, we were still interested in developing a synthesis technique that will produce a material with a more exact charge balance. As we mentioned in our previous study,<sup>6b</sup> the grafting procedure is theoretically better suited for the synthesis of zwitterionic stationary phases with good charge balance, because the material results from a monomer with an exact charge balance.

In the current study, we have synthesized a zwitterionic stationary phase designated KS–TC–TBHP–SPE by grafting 3-dimethyl(methacryloyloxyethyl) ammonium propanesulfonate (SPE) onto porous spherical silica, using *tert*-butyl hydroperoxide (TBHP) attached to a thionyl chloride (TC) activated Kromasil silica (KS) surface to induce the polymerization. This procedure causes the grafting to start from the particle surface. The charge balance between quaternary and sulfonic groups in the graft layer was determined by elemental analysis. The chromatographic evaluation involved packing the material into a column on which small inorganic ions and proteins were successfully separated.

## EXPERIMENTAL SECTION

**Reagents and Chemicals.** Kromasil spherical silica particles (10- $\mu$ m particle size with 200-Å pore size) were obtained from EKA Chemicals (Bohus, Sweden). The zwitterionic monomer 3-[*N,N*-dimethyl-*N*-(methacryloyloxyethyl)ammonium] propanesulfonate

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(SPE) [3637-26-1] was obtained from Raschig Chemie (Ludwigshafen, Germany), and *tert*-butyl hydroperoxide (5 M in octane) was purchased from Aldrich (Steinheim, Germany). The zwitterionic amphiphile used for comparative  $\zeta$ -potential measurements in combination with Nucleosil C<sub>18</sub> (3  $\mu$ m; 120 Å; Macherey-Nagel, Düren, Germany) was *N*-dodecyl-*N,N*-dimethyl-3-ammonio-1-propanesulfonate (SB12; Aldrich). Thionyl chloride (99%) was obtained from Fluka (Buchs, Switzerland), and methanol (HPLC grade) was from J. T. Baker (Deventer, Holland). Perchloric acid (70%, p.a.) was from Riedel de Haën (Seelze, Germany). Acetone (purum) for washing was from Svenda AB (Stockholm, Sweden), and chloroform (p.a.) from Prolabo (Fontenay S/Bois, France). Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA). The inorganic salts used in this paper were the same as those used in the previous papers.<sup>6</sup> Stock solutions of salts for injection were prepared by dissolving the salts directly in Milli-Q water. Proteins used as test solutes were purchased from Sigma (St. Louis, MO) and were the following (CAS number; Sigma product number): ovalbumin from chicken egg (9006-59-1; A5503); conalbumin from chicken egg (1391-06-6; C7786);  $\alpha$ -chymotrypsinogen A type II, from bovine pancreas (9035-75-0; C4879); cytochrome *c*, from horse heart (9007-43-6; C7752); and lysozyme, from chicken egg white (12650-88-3; L6876). All purchased proteins were stored according to the manufacturer's recommendations and used as received to prepare solutions for injection by dissolution in water.

**Synthesis and Characterization of the KS–TC–TBHP–SPE Zwitterionic Stationary Phase.** A 5-g aliquot of Kromasil silica particles was dried at 110 °C for 8 h under vacuum. The dried silica was then transferred to a mixture of 75 mL chloroform and 75 mL thionyl chloride and refluxed for 18 h under slow stirring. The suspension was then allowed to cool to room temperature, filtered on a glass filter, and washed with large amounts of methanol and acetone. The collected activated silica particles were then transferred to a 100-mL E-flask, charged with 70 mL of 1,4-dioxan, 20 mL *tert*-butyl hydroperoxide solution, and 0.25 g of solid sodium bicarbonate. The flask was covered with aluminum foil and reacted on a shaker at room temperature for 18 h. The peroxidated particles were washed with methanol and dried at room temperature under vacuum.

Two grams of the peroxide-functionalized silica were added to a 100-mL three-neck round-bottom flask. To this flask was charged 60 mL of water, 15 mL of methanol, and 10 g of SPE zwitterionic monomer. The mixture was thereafter degassed with nitrogen gas for 30 min. The graft polymerization was carried out at 80 °C for 9 h with slow stirring under nitrogen. Finally, the polymerization suspension was mixed with  $\approx$ 250 mL of 1 M aqueous sodium chloride and ultrasonicated for 5 min, after which the particles were allowed to settle, and the solution was decanted. Resuspension in aqueous NaCl was repeated several times, followed by careful washing with water, filtering, and drying in air. The sulfur and nitrogen contents of the intermediates and the final material were determined by elemental analysis by MikroKemi AB (Uppsala, Sweden) using validated methods.

The  $\zeta$ -potentials were measured by photon correlation spectroscopy using a Malvern (Malvern, U.K.) Zetasizer 4 instrument. Stock samples were prepared by suspending 50 mg of material in 30 mL of water. These stock samples were resuspended prior

Table 1. Elemental Analysis of Silica before and after Grafting with Zwitterionic Brushes

material	% N <sub>EA</sub> <sup>a</sup>	% S <sub>EA</sub> <sup>a</sup>	% S <sub>calc</sub> <sup>b</sup>	% S <sub>EA</sub> / % S <sub>calc</sub> <sup>c</sup>
activated for grafting <sup>d</sup>	0.16	<0.20 <sup>e</sup>	N/A <sup>f</sup>	N/A
KS–TC–TBHP–SPE	1.07	2.47	2.45	1.01:1

<sup>a</sup> Percent N and S determined from elemental analysis. <sup>b</sup> Stoichiometric % S calculated from N<sub>EA</sub> according to the molar weight ratio of 32.06:14.01. <sup>c</sup> Amount of S incorporated in relation to the stoichiometric ratio. <sup>d</sup> Particles with peroxide groups that were prepared for the final grafting step. <sup>e</sup> The sulfur content was below the detection limit. <sup>f</sup> N/A, not applicable.

to each determination, and the final samples were prepared by mixing 1 mL of stock sample with buffer, salt, or both at the appropriate concentration, then were diluted with water if necessary. The final volume was 6 mL in the experiments presented in Table 2 and 10 mL for those in Table 3. After a solution was made up, it was thoroughly mixed on a Heidolph (Schwabach, Germany) REAX Control vortex mixer and immediately thereafter transferred to the measurement cell. The coating procedure for the Nucleosil C<sub>18</sub> took place by suspending a 50-mg aliquot in 30 mL of water to which had been added 100 mg of SB12. The suspension was thereafter treated in a Branson (Danbury, CT) model 3510 ultrasonic bath for 30 min and shortly thereafter subjected to the  $\zeta$ -potential determination. The sample was treated as described above, and no attempts were made to remove the unadsorbed SB12 from the coating solution. Reported  $\zeta$ -potential values are mean  $\pm$  standard deviation for three repeated measurements on the same sample, made in rapid succession.

The column back-pressures were measured at room temperature (22  $\pm$  2 °C) with the built-in digital pressure indicator of a Spectra-Physics (Mountain View, CA) model SP8700XR HPLC pump. After each change of solution, the column was equilibrated at a flow rate of 1.00 mL/min until the back-pressure remained stable within  $\pm$ 2 psi ( $<\pm$ 0.4% of the absolute pressure) over 10 min, after which the readout value was recorded. The pressure indicator was not calibrated, but the linearity of the pump delivery rate/readout system vs the programmed flow rate was ascertained by plotting the readout as function of five set flow rates over the interval 0–1 mL/min, whereby a linear, zero-crossing function was obtained.

**Column Packing and Chromatographic Evaluation.** The silica particles with zwitterionic stationary functional groups were slurry-packed into 150-mm-long  $\times$  4-mm-i.d. poly(ether-ether-ketone) (PEEK) column blanks from Upchurch Scientific (Oak Harbor, WA), using Milli-Q water as packing solvent. The packing was accomplished with a high flow pump of the pneumatic amplifier type (Knauer, Berlin, Germany) using a final hydraulic pressure of 60 MPa.

The chromatographic system used for evaluating the protein separation consisted of an SP 8700XR gradient LC pump, an AS3000 autosampler with a 20- $\mu$ L stainless steel injection loop, and a UV 150 UV spectrophotometric detector (all from Spectra-Physics). In the evaluation of small inorganic ions, the detector was replaced by an LDC (Laboratory Data Control, Riviera Beach, FL) Conducto-Monitor electrolytic conductivity detector with a Conductocell 7011. All chromatograms were recorded on a Star

Table 2. Results from the  $\zeta$ -Potential Measurements on the Material KS–TC–TBHP–SPE

pH	phosphate buffer		10 mM phosphate buffer + NaCl			10 mM phosphate buffer + NaClO <sub>4</sub>		
	10 mM	90 mM	2 mM	20 mM	80 mM	2 mM	20 mM	80 mM
5.0	−8.6 ± 4.1	−8.9 ± 0.6	−11.3 ± 0.9	−10.2 ± 0.8	−9.1 ± 0.7	−13.4 ± 3.9	−16.1 ± 1.5	−20.4 ± 3.2
6.0	−13.1 ± 1.4	−9.9 ± 0.4	−12.4 ± 1.2	−13.0 ± 0.8	−9.0 ± 0.7	−11.3 ± 0.8	−15.7 ± 1.7	−20.5 ± 0.4
7.0	−14.7 ± 1.0	−10.7 ± 0.6	−15.8 ± 1.1	−12.2 ± 0.8	−9.5 ± 1.1	−12.2 ± 0.3	−16.4 ± 0.1	−22.3 ± 0.6
8.0	−12.8 ± 0.8	−12.6 ± 0.7	−13.1 ± 0.4	−12.7 ± 0.6	−12.2 ± 0.7	−11.7 ± 1.1	−14.9 ± 1.8	−20.9 ± 1.6

Table 3. Back-pressure for the KS–TC–TBHP–SPE Column with Different Acids and Salts

concn, mM	Change in back-pressure relative to water, %					
	HCl	HNO <sub>3</sub>	HClO <sub>4</sub>	NaCl	NaNO <sub>3</sub>	NaClO <sub>4</sub>
2	−2	−2	1	3	4	4
20	−1	−1	23	3	4	24

workstation (Varian, Palo Alto, CA). The chromatographic evaluations were carried out at room temperature (22 ± 2 °C).

## RESULTS AND DISCUSSION

**Synthesis and Characterization of the Zwitterionic Stationary Phase KS–TC–TBHP–SPE.** The schematic procedure for the synthesis of the grafted KS–TC–TBHP–SPE material is shown in Scheme 1. The silica particles were first dehydrated with thionyl chloride to obtain silicone chloride reactive groups, followed by a reaction with *tert*-butylhydroperoxide to attach the peroxide groups. Finally, the grafting polymerization was started from a suspension of the modified particles in SPE. The reason we chose this method was to ascertain that the initiating groups were covalently bonded to the surface of the matrix particles. Therefore, free radical polymerization initiation sites will be created on the surface when the particles are heated to trigger the thermally initiated polymerization. Although a corresponding amount of *tert*-butoxy free radicals will be released into the solution by cleavage of the surface-bound initiator groups, the concentration in solution will be considerably lower compared to the radical concentration used for grafting initiated by a solution-phase free radical source. Tsubokawa and Ishida developed this chemistry for the surface modification of ultrafine silica 10 years ago,<sup>7</sup> but this grafting scheme does not appear to have been utilized since then. This is therefore a new way of preparing tentacle-type sorbents for chromatographic purposes through surface-initiated grafting.

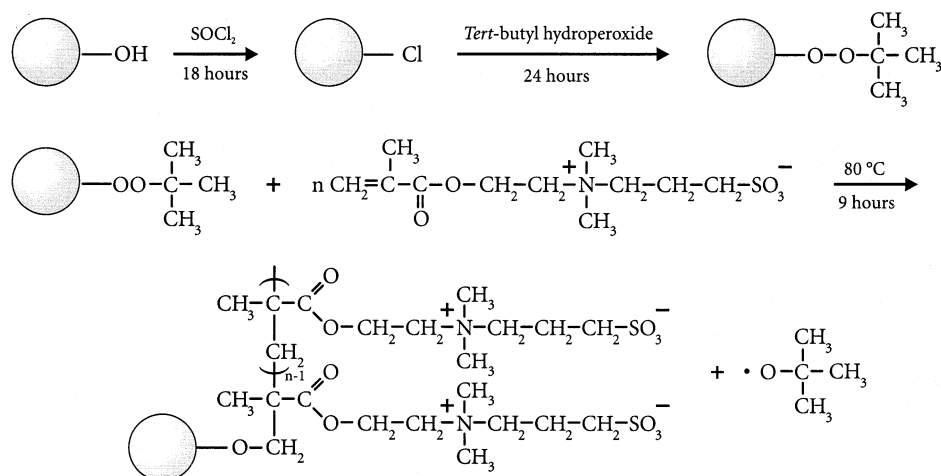
According to the elemental analysis result in Table 1, the molar ratio of nitrogen to sulfur was 1:1.01. This means the grafted zwitterionic material had an exact charge balance. The surface charge properties were then studied by particle electrophoresis in a Zetasizer photon correlation spectrometer, where the  $\zeta$ -potentials for the particles were measured in 10 mM aqueous phosphate buffer at four different pH levels, with or without the addition of sodium chloride and sodium perchlorate. The results of these measurements are presented in Table 2, showing insignificant changes in  $\zeta$ -potential as a function of pH with buffer only, and with sodium chloride added to the buffer. However, the addition of perchlorate ions to the buffer caused a decrease in the  $\zeta$ -potentials, which became increasingly negative as the perchlorate concentration was increased. This trend was evident

at all the pH levels tested. This induction of a more negative surface charge is in accordance with the effects of perchlorate that our group<sup>6,8</sup> and other research groups<sup>9,10</sup> have observed earlier. Attempts were also made to measure the  $\zeta$ -potentials in water, but the values obtained were not stable. The measurements were also difficult at the lowest salt concentration. The slurry had to be prepared immediately prior to filling the measurement cell, and the measurements had to be conducted rapidly because of aggregation and settling of the particles. It should be kept in mind that SPE homopolymer has an “antipolyelectrolytic” behavior in aqueous solution;<sup>11</sup> i.e., the solubility of the linear zwitterionic polymer increases with increasing salt concentration, a salt-dependent solubility relationship that is opposite to conventional polyelectrolytes. The tendency of the particles to aggregate at low ionic strength and their failure to disperse in pure water, therefore, reveal the tentacle nature of the grafted layer. The presence of a grafted layer is further verified by the salt-dependent back-pressure of the column (Table 3). When operated in pure water or with NaCl, NaNO<sub>3</sub>, and 2 mM HClO<sub>4</sub>, the back-pressure was ≈3750 kPa. When 20 mM perchloric acid or sodium perchlorate was pumped, the back-pressure rose to ≈4600 kPa. This increase in back-pressure took place in the concentration interval where our previous study<sup>6a</sup> showed that the sulfopropylbetaine groups have their maximum uptake of HClO<sub>4</sub>. A significant back-pressure increase that cannot be explained by a salt-induced viscosity change of the eluent was also seen during gradient elution from 0 to 1 M NaCl in 10 mM phosphate buffer. This increased flow resistance in the presence of salts can be explained by the viscous drag experienced by the eluent as the grafted chains extend into the flow paths.

Measurements were then made of the  $\zeta$ -potentials of native silica and silica modified with zwitterionic groups by grafting and dynamic equilibration (cf. Table 4). The bare silica had a surface potential close to 0 in the tested acids at both the concentration levels, and the type of anion had little effect. Its  $\zeta$ -potential became slightly negative in neutral solution (salt only), and there were no discernible potential differences for sodium salts with different anions. When comparing the  $\zeta$ -potentials measured in 2 and 20 mM salt solutions, it was found that the  $\zeta$ -potential increased at the higher concentration level, which is expected due to suppression of the double layer.

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Scheme 1. Synthesis Steps Used for Preparing the Zwitterionic Stationary Phase KS-TC-TBHP-SPE

Table 4.  $\zeta$ -potentials of Silicas Modified with Zwitterionic Groups by Grafting and Dynamic Coating

material <sup>a</sup>	HCl	HNO <sub>3</sub>	HClO <sub>4</sub>	NaCl	NaNO <sub>3</sub>	NaClO <sub>4</sub>
	2 mM	2 mM	2 mM	2 mM	2 mM	2 mM
KS	3.6 ± 1.1	4.5 ± 2.9	2.3 ± 1.6	-12.6 ± 0.3	-11.4 ± 7.3	-13.6 ± 2.5
KS-TC-TBHP-SPE	1.0 ± 0.4	0.9 ± 2.3	-8.1 ± 0.6	-15.4 ± 3.9	-13.7 ± 7.0	-25.4 ± 6.0
Nucleosil-ODS-SB12	-13.4 ± 0.4	-16.5 ± 0.5	-44.2 ± 0.9	-17.5 ± 1.1	-34.5 ± 1.0	-37.6 ± 1.3
	20 mM	20 mM	20 mM	20 mM	20 mM	20 mM
KS	2.9 ± 0.3	2.3 ± 0.5	0.2 ± 0.2	-6.8 ± 4.0	-4.4 ± 2.0	-5.5 ± 2.3
KS-TC-TBHP-SPE	-6.4 ± 0.5	-9.8 ± 2.5	-16.0 ± 1.3	-8.3 ± 2.4	-10.9 ± 1.4	-12.2 ± 2.7
Nucleosil-ODS-SB12	-12.2 ± 0.6	-22.5 ± 0.4	-39.3 ± 0.3	-11.6 ± 0.3	-25.0 ± 0.5	-49.7 ± 1.1

<sup>a</sup> KS, Kromasil silica; KS-TC-TBHP-SPE, KS grafted with tentacle zwitterionic polymer chains; Nucleosil-ODS-SB12, Nucleosil C<sub>18</sub> coated with SB12.

For the SPE grafted silica, the  $\zeta$ -potential in both acids and salts of three different anions showed the same general trend. At the lower concentration level tested, there was no difference between the  $\zeta$ -potentials in HCl and HNO<sub>3</sub>, nor between NaCl and NaNO<sub>3</sub>. However, with HClO<sub>4</sub> and NaClO<sub>4</sub>, the  $\zeta$ -potentials were more negative than with the two other acids and salts. The same anion-dependent pattern was apparent at the higher concentration, although the  $\zeta$ -potentials became lower in the acids and higher in the salt solutions; i.e., the surface charge density increased with an increasing acid concentration and decreased with an increasing concentrations of neutral salts. Overall, the  $\zeta$ -potentials of the SPE-grafted silica were of substantially lower magnitude than those of the Nucleosil C<sub>18</sub> that had been coated with a sulfoalkylbetaine detergent. This reveals that the grafted material had a substantially lower surface charge density, despite the thickness of the grafted layer evident from the elemental analysis data in Table 1 and the increase in back-pressure seen at the high concentration levels of perchloric acid and sodium perchlorate in Table 3. A lower surface charge density is expected when the grafted chains expand as a result of increasing acid or salt concentration due to "dilution" of the net charge in the extended layer.<sup>12</sup> For sodium perchlorate, this was observed as a lower absolute value for the  $\zeta$ -potential, but for perchloric acid, the  $\zeta$ -potential magnitude doubled as the acid concentration was increased from 2 to 20 mM. We have no explanation for this

behavior at present, but we recollect that a polymer-based sulfoalkylbetaine material we reported on earlier<sup>6a</sup> had a much higher affinity for perchloric acid than for sodium perchlorate.

As mentioned, the changes in  $\zeta$ -potentials of the Nucleosil-ODS-SB12 material were considerably larger than those seen with the grafted material. At both of the concentration levels tested, there was a trend toward more negative potential with anion type in the series Cl<sup>-</sup> < NO<sub>3</sub><sup>-</sup> < ClO<sub>4</sub><sup>-</sup>, indicating that the association of the more chaotropic anions was more pronounced than on the grafted material. This can again be an effect of the high density of the zwitterionic groups in the absorbed layer on the Nucleosil-ODS-SB12 compared to the charge density expressed by the comparatively sparse layer of SPE chains grafted onto the bare silica.

**Effect of Mobile Phase Concentration on the Separation of Inorganic Anions and Cations.** The salts Na<sub>2</sub>SO<sub>4</sub>, NaCl, NaBr, NaNO<sub>3</sub>, NaI, and NaSCN were injected individually at 1 mM concentration onto the packed column, using perchloric acid as eluent at concentrations ranging from 1 to 10 mM. The general tendency was that the retention times of all anions decreased with increasing perchloric acid concentration, whereas the retention dependence of the sodium ion was the opposite (Figure 1). This retention pattern is similar to those we have observed for materials of good charge balance in previous studies of surface-modified and grafted zwitterionic stationary phases based on polymeric substrates, most notably the surface-modified material termed S300-TC-DMA-PS in ref 6. That material shares with the current

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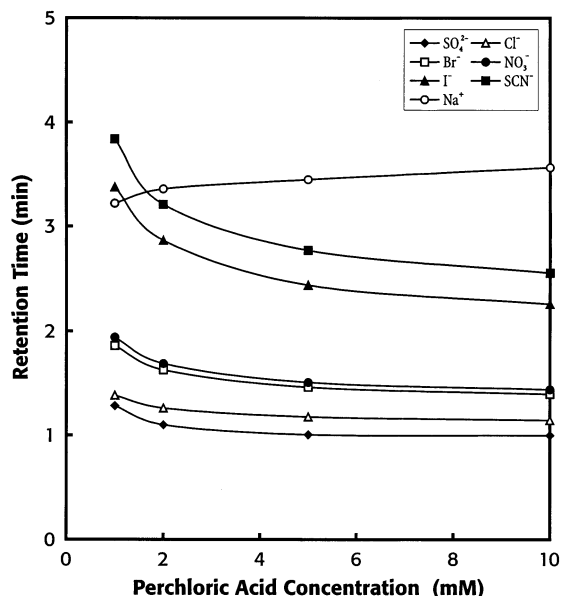


Figure 1. Effect of perchloric acid concentration on the retention time of inorganic anions and cations on the KS–TC–TBHP–SPE column with aqueous perchloric acid as eluent at 1 mL/min. Direct conductivity detection was used in all experiments.

silica-based grafted material a low overall retention for anions, with an eluent concentration dependence that does not reflect the conventional stoichiometric ion exchange retention model for ions on fixed-charge ion exchangers; i.e.,  $\log(k')$  vs  $\log[\text{eluent concentration}]$  does not describe a linear relationship with a slope whose absolute value is equal to the quotient of the charges of the elute ion and the counterion involved in the elution process.<sup>13</sup> Elemental analyses revealed that a capacity of 0.76 mmol/g was reached, which is lower than the capacities we attained on polymeric substrates. The retentiveness of the silica-based covalently bonded zwitterionic exchanger was also lower than any of the materials we have reported earlier<sup>6</sup> and considerably lower than conventional, singly charged ion exchangers of similar functional group density.

#### Simultaneous Separation of Inorganic Anions and Cations.

To compare the chromatographic properties of the new silica-based grafted zwitterionic material with the polymeric materials published in our previous papers, a mixture with several different inorganic anions and cations was prepared and injected onto the column. It was found that cations and anions could be simultaneously separated on the basis of their individual properties using eluents-based magnesium perchlorate, as shown in Figure 2. Perchloric acid was also used as the eluent to separate the same mixture as above, but  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  could not be eluted within 30 min. This should be because the sulfonic groups strongly retain the divalent cations, and the eluent was not strong enough to elute them in a reasonable time. As we mentioned in one of our previous studies,<sup>6b</sup> the magnesium ions enhance the retention of anions due to their interaction with the sulfonic group of the zwitterionic group, causing a lower negative charge on the material. Furthermore, since magnesium is a strong counterion, it can elute  $\text{Ca}^{2+}$  from the column in a reasonable time. However, a problem with

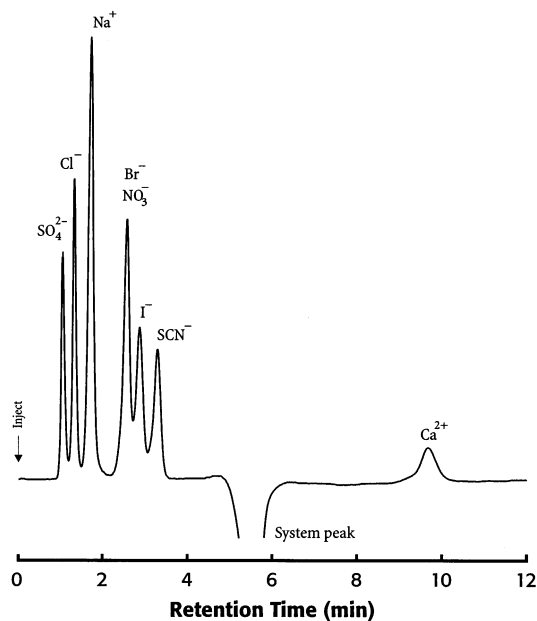


Figure 2. Chromatogram from the separation of a mixture containing 1 mM  $\text{CaSO}_4$ ,  $\text{CaCl}_2$ ,  $\text{Ca}(\text{NO}_3)_2$ , and 2 mM  $\text{NaBr}$ ,  $\text{NaI}$ , and  $\text{NaSCN}$  on the KS–TC–TBHP–SPE column using 5 mM magnesium perchlorate at a flow rate of 1 mL/min as eluent. Direct conductivity detection was used in the experiment.

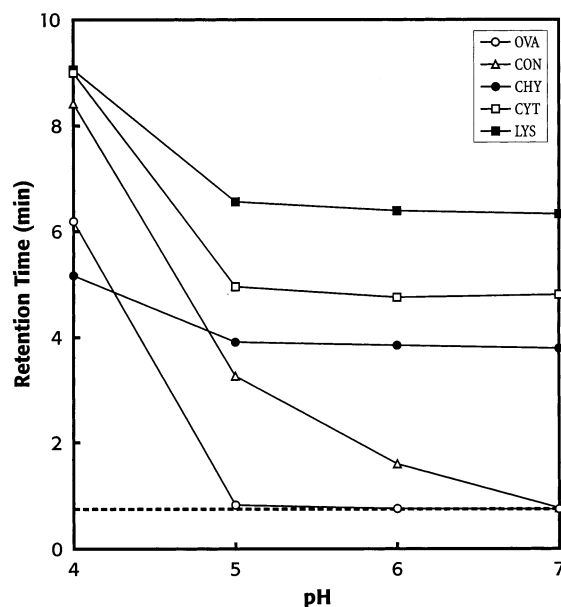


Figure 3. Effect of pH on the retention of proteins on the KS–TC–TBHP–SPE column. The proteins were injected as a mixture in water containing 2 mg/mL of ovalbumin (OVA),  $\alpha$ -chymotrypsinogen A (CHY), cytochrome c (CYT), and lysozyme (LYS), and 4 mg/mL of conalbumin (CON). Elution was accomplished by a gradient from 100% A to 100% B in 15 min at a flow rate of 1 mL/min. Eluent A, 10 mM phosphate buffer at the pH indicated; eluent B, 10 mM phosphate buffer plus 1 M  $\text{NaCl}$ . The dashed horizontal line corresponds to the dead time in the chromatographic system.

the present material is that nitrate and bromide ions overlap in the chromatograms when magnesium perchlorate is used as eluent.

**Effect of pH on the Separation of Acidic and Basic Proteins with Gradient Elution.** In addition to the separation of small inorganic ions, the grafted silica can also be used for the

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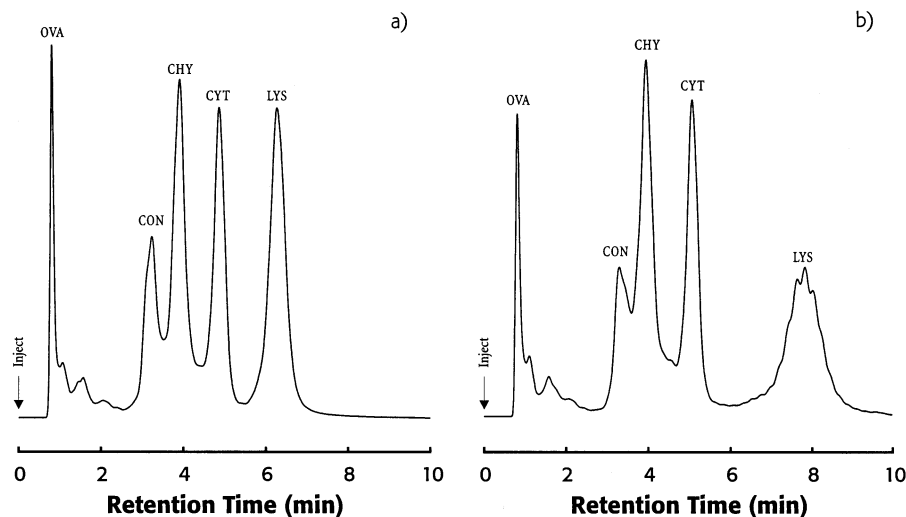


Figure 4. Chromatograms of a mixture of proteins with a composition identical to that used in Figure 3. Gradient elution: from 100% A to 100% B in 15 min at a flow rate of 1 mL/min starting at the time of injection. Eluent A, 10 mM, pH 5.3 phosphate buffer; (a) eluent B, 10 mM, pH 5.3 phosphate buffer plus 1 M NaCl; (b) eluent B, 10 mM, pH 5.3 phosphate buffer plus 1 M NaCl and 0.1 M NaClO<sub>4</sub>.

separation of proteins. Figure 3 shows the effect of pH on the retention times of ovalbumin (4.7;<sup>13</sup> 4.9<sup>14</sup>), conalbumin (6.0, 6.3, 6.6),<sup>13</sup>  $\alpha$ -chymotrypsinogen A (8.8, 9.2, 9.6;<sup>13</sup> 9.1<sup>15</sup>), cytochrome *c* (9.0, 9.4),<sup>13</sup> and lysozyme (11;<sup>13</sup> 10.7<sup>14</sup>) (pIs in parentheses), the protein probes used in this study. It can be seen that the retention times of these proteins decreased with increasing pH of the buffer solution. Ovalbumin and conalbumin lacked retention at pH above 5 and 7, respectively. Because the  $\zeta$ -potential remained almost unchanged in four tested pH ranges, we therefore attribute this to a dissociation of acidic groups on the protein surface, which induces a more negative charge as the eluent's pH is increased. Since the cation exchange properties are prevailing due to the distal position of the sulfonic acid moiety in the sulfopropylbetaine group, the retention will disappear as the protein alters its overall charge from positive to negative.

#### Simultaneous Separation of Acidic and Basic Proteins.

A simultaneous separation of acidic and basic proteins was finally attempted, using gradient elution from 10 mM phosphate buffer, pH 5.3, to the same buffer containing salt. Two different salt buffers were used as the stronger eluent: one containing 1 M NaCl and the other, 1 M NaCl and 0.1 M NaClO<sub>4</sub>. Typical chromatograms are shown in Figure 4. Ovalbumin eluted in the void volume with both gradient eluents as a result of the low pI of the protein and a negative surface charge of the zwitterionic stationary phase. When parts a and b of Figure 4 are compared, it can be seen that the retention times for conalbumin, chymotrypsinogen A, cytochrome *c*, and lysozyme increased when perchlorate ion was added to the stronger eluent. In accordance with our previous studies,<sup>6,8,9</sup> the chaotropic perchlorate ion interacts preferentially with the quaternary ammonium group, thus

inducing a more negative surface potential on the zwitterionic material. Although the observed  $\zeta$ -potential decrease (Table 2) was less pronounced than those of the organic-polymer-based materials that we described earlier,<sup>6</sup> the addition of perchlorate ions still affected the ion exchange character of the zwitterionic phase, an effect manifest in retention time increases in buffers containing perchlorate ion. Modulation of the tenacity of the zwitterionic stationary phase by addition of perchlorate ions is, thus, possible also on the present separation medium, on the basis of graft polymerized zwitterionic chains on silica instead of copolymerized<sup>8a</sup> or postpolymerization-modified<sup>8b</sup> polymeric zwitterionic sorbents.

#### CONCLUSION

A new zwitterionic stationary phase was synthesized by graft polymerization of the zwitterionic monomer SPE onto the surface of porous silica particles. The resulting material had a unity charge balance and showed separation features typical of zwitterionic stationary phase. It was capable of separating inorganic cations and anions simultaneously using aqueous solution of perchloric acid or perchlorate salts as eluent and could also separate acidic and basic proteins in a single run using gradient elution.

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