



## Polyethylenimine-modified metal oxides for fabrication of packed capillary columns for capillary electrochromatography and capillary liquid chromatography

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### ARTICLE INFO

#### Article history:

Available online 23 December 2010

#### Keywords:

Capillary liquid chromatography  
Capillary electrochromatography  
Metal oxides  
Polyethylenimine functionalized metal oxides  
Nanocasting  
Slurry packing

### ABSTRACT

The need for novel packing materials in both capillary electrochromatography (CEC) and capillary liquid chromatography (CLC) is apparent and the development towards more selective, application-oriented chromatographic phases is under progress world-wide. In this study we have synthesized new polyethylenimine (PEI) functionalized  $\text{Mn}_2\text{O}_3$ ,  $\text{SiO}_2$ ,  $\text{SnO}_2$ , and  $\text{ZrO}_2$  particles for the fabrication of packed capillary columns for CEC and CLC. The nanocasting approach was successful for the preparation of functionalized metal oxide materials with a controlled porosity and morphology. PEI functionalization was done using ethyleneimine monomers to create particles which are positively charged in aqueous solution below pH 9. This functionalization allowed the possibility to have both hydrophobic (due to its alkyl chain) and ionic interactions (due to positively charged amino groups) with selected compounds. For comparison aminopropyl-functionalized silica was also synthesized and tested. Both slurry pressure and electrokinetic packing procedures used gave similar results, but fast sedimentation of the material caused some problems during the packing. The high stability and wide pH range of PEI-functionalized  $\text{SiO}_2$  material, with potential for hydrophobic and electrostatic interactions, proved to be useful for the CEC and CLC separation of some model acidic and neutral compounds.

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### 1. Introduction

Chromatography is already a well established technique in industry and at research institutions (public/state) and both instruments and new stationary phases are continuously under development. However, even though new commercial stationary phases are developed and marketed world-wide, there is still a great need for novel packing materials towards more selective, application-oriented chromatographic separations. To date, silica-based reversed phase columns completely dominate HPLC. The silica surface chemistry and surface modification reactions are well understood, as are the limitations of silica. The introduction of the new generation hybrid silica-based columns and polymeric reversed phases has allowed chromatographic separations to be carried out at almost the whole pH range. On the other hand, the

drawbacks of polymeric phases still exist, such as swelling and quite low mechanical stability and separation efficiency. The invention and development of monolithic columns can be seen as another great landmark in the development of LC during the last century.

There have been several attempts to circumvent some limitations of silica-based materials, and oxides of zirconium, titanium, alumina, and to a lesser extent thorium and cerium, have been tested, of which zirconium oxide-based materials have provided the best results [1–6]. The stability of commercial metal oxide phases is so high that the stationary phase is no longer the limiting factor of the chromatographic system instead the weakest link seems to be the column hardware. However, the production of hydrophobically modified metal oxide surfaces is more challenging. Commercial zirconia-based LC columns have mainly been useful for the separation of low-molar mass analytes [7]. Silica particles (5  $\mu\text{m}$ ) have been directly modified with cobalt, nickel, zinc, and cadmium salts and used as packing materials for normal-phase HPLC [8]. Zirconia-coated silica monoliths have been prepared and used in CEC [9] as well as pure titania-modified

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monoliths that have been used for hydrophilic interaction liquid chromatographic (HILIC) separation of xanthenes [10]. Recently, sol–gel reactions were used to prepare TiO<sub>2</sub> monolithic chromatographic columns [11]. There is much research going on related to metal oxide monoliths, however, special attention has been paid to the characterization of materials (see, e.g. [12–14] and refs therein). Immobilized metal ion affinity chromatography (IMAC) type chromatographic stationary phases have already matured for enrichment, purification, and analysis of phosphopeptides and phosphoproteins (see, e.g. [15–17] and refs therein) and there are several commercial protocols and kits available. Metal oxide affinity chromatography (MOAC), using TiO<sub>2</sub>, ZrO<sub>2</sub>, and SnO<sub>2</sub>, has also successfully been used in the enrichment of phosphopeptides [18–20].

When synthesizing new packing material one limiting factor is the amount of material obtained. The low amount of purified material puts great demands on the preparation of columns. This is one of the reasons why the suitability of the materials as stationary phases is tested in capillary liquid chromatography (CLC) [21]. Such packed capillaries can subsequently be utilized in capillary electrochromatography (CEC) [22,23]. In this work we have synthesized novel polyethyleneimine (PEI) modified metal oxide materials to be used as packing materials for CLC and CEC. Ethyleneimine (aziridine) was used to generate a covalently bonded PEI functionalization, which is positively charged in aqueous solutions at pH values lower than 9 [24]. Compared to conventional physical adsorption of PEI polymers, this approach offers a more chemically stable dendrimer structure of PEI on the metal oxide surface. PEI-functionalization was done in order to have both electrostatic and hydrophobic interactions with selective compounds. Direct PEI-modification of fused-silica capillaries has frequently been used in chromatography [25] (and refs therein). The materials investigated in this work included 5 μm PEI-functionalized SiO<sub>2</sub>, ZrO<sub>2</sub>, Mn<sub>2</sub>O<sub>3</sub>, and SnO<sub>2</sub> particles (porosity of 120 Å).

## 2. Experimental

### 2.1. Apparatus and instruments

A Jeol JSM-6335F scanning electron microscope (SEM; Jeol, Tokyo, Japan) was used for the study of the materials morphology. Nitrogen physisorption measurements were performed using an accelerated surface area and porosimetry system (ASAP 2010) from Micromeritics (Norcross, GA, USA). A thermogravimetric analyzer TGA 209 and a differential scanning calorimeter STA449 from Netzsch (Selb, Germany) were used for the determination of the degree of functionalization of the different oxide samples. A Malvern Zeta-Sizer Nano-ZS from Malvern Instruments (Malvern, UK) was used for characterization of the electrokinetic properties of the particles.

Uncoated fused-silica capillaries from Polymicro Technologies (Phoenix, AZ, USA) with inner diameters of 100 μm and outer diameters of 360 μm were used throughout the study. PEI-functionalized SnO<sub>2</sub>, ZrO<sub>2</sub>, or Mn<sub>2</sub>O<sub>3</sub> particles were packed also in 3-aminopropyltriethoxysilane (APTES) and polyacrylamide coated capillaries, prepared as described [30]. A Jasco PU-980 LC-pump was used for packing and flushing the capillaries. The success of the packing procedure was controlled by an optical microscope of AlphaPhot-2 YS2 model (Nikon, Tokyo, Japan).

CEC measurements were made with a Hewlett-Packard <sup>3D</sup>CE system (Agilent, Waldbronn, Germany) equipped with a diode array detector (detection at 200 and 254 nm), air thermostated capillary and with the capability to apply up to 12 bar pressure to both ends of the capillary was employed in the experiments. The data acquisition rate was 100 Hz and the response time of the detector 0.1 s.

CLC runs were performed by two different instruments, of which one was commercial and one a home-built device. The commercial instrument was an Ultimate Chromatographic System Ultimate 3000 device from Dionex (Sunnyvale, CA, USA), that was furnished with an auto sampler from Dionex and an on-column single-wavelength UV CE-Quanta 4000 (Milford, MA, USA) detector from Waters. The home built device comprised of a PU-980 high-pressure LC pump from Jasco (Easton, MD, USA), a 1:1000 flow splitter from Valco (Houston, TX, USA), a low dispersion microscale injector 7410 from Rheodyne (Oak harbor, WA, USA), and an on-column single wavelength UV CE-Quanta 4000 detector from Waters. The effective in-column flow rate was experimentally estimated by using a micro syringe (10 μL) joined to the outlet column by a Teflon tube (TF-350; LC Packing, CA, USA). The mobile phase was collected for 5 min measuring the exact volume.

### 2.2. Materials

5 μm mesoporous silica spheres (chromatography beads) with a pore size of about 120 Å were obtained as a gift from Shiseido, Tokyo, Japan, and were used as received. ODS material (C18 end-capped 3 μm silica, porosity 8 nm) was kindly donated by Professor Peter Myers of Xtec (Department of Chemistry, University of Liverpool, Liverpool, UK). PEI-functionalized SiO<sub>2</sub>, ZrO<sub>2</sub>, SnO<sub>2</sub>, and Mn<sub>2</sub>O<sub>3</sub> as well as aminopropyl-functionalized SiO<sub>2</sub> were synthesized by a nanocasting process and tested by CEC and CLC. In the nanocasting process ZrOCl<sub>2</sub>·8H<sub>2</sub>O (99%, Fluka Chemie GmbH, Buchs, Switzerland), SnCl<sub>2</sub> (98%, Riedel-de Haën, Seelze/Hannover, Germany), and Mn(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O (97%, Fluka) were used as metal oxide precursors, with either de-ionized water (MilliQ, Millipore) or ethanol (99.5%) as solvents. APTES (98%, Sigma–Aldrich, St. Louis, MO, USA) was used to change the negative inner capillary surface to cationic. Ethyleneimine (aziridine) was synthesized from aminoethylsulphuric acid using a procedure described by Allen et al. [26]. In short, aminoethylsulphuric acid (Aldrich, Stenheim, Germany) (100 g) was mixed with a sodium hydroxide solution (400 mL of 14%, w/v NaOH in water) and heated to boiling point. The bath was removed after boiling started and resumed as the mixture started to cool. About 100 mL of distillate boiling between 50 °C and 105 °C was quickly collected. Potassium hydroxide (KOH) was added to the cooled distillate, whereas ethyleneimine separated as an upper layer. Ethyleneimine was then separated from the mixture and stored over KOH at 4 °C overnight. After observing that no aqueous layer appeared, the imine was decanted and distilled from KOH pellets yielding a clear colorless liquid boiling at 55–57 °C. Note: ethyleneimine is an extremely hazardous chemical and all handling should be carried out with appropriate precaution.

Tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl) and phosphoric acid (Sigma–Aldrich) were used for preparation of the buffer solutions used as BGE or mobile phase in CEC and CLC, respectively. The pH of the solutions was adjusted by addition of sodium hydroxide or hydrochloric acid from FF-Chemicals (Yli Ii, Finland). Methanol was used as solvent for slurry preparation and as a packing carrier.

Acetone, phenol, benzene, benzoic acid, 2-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 2,3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, and 2,6-dihydroxybenzoic acid (from Sigma–Aldrich) were used as model compounds for characterization of the different packed materials.

### 2.3. Preparation of ZrO<sub>2</sub>, SnO<sub>2</sub>, and Mn<sub>2</sub>O<sub>3</sub> particles using the nanocasting process

The ZrO<sub>2</sub>, SnO<sub>2</sub>, and Mn<sub>2</sub>O<sub>3</sub> spheres used in this study were prepared according to the protocol described by Smått et al. [27]. In the preparation of the ZrO<sub>2</sub> and SnO<sub>2</sub> replicas, ZrOCl<sub>2</sub>·8H<sub>2</sub>O and SnCl<sub>2</sub>

were used as precursors. The  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$  and  $\text{SnCl}_2$  concentrations used were  $1.0 \text{ g mL}^{-1}$  and  $1.8 \text{ g mL}^{-1}$ , respectively. After an impregnation time of 1 h, centrifugation was used to sediment the particles and then the excess precursor solution was removed. The samples were directly heated at  $150^\circ\text{C}$  (3 h) to remove the solvent and further heated up to  $250^\circ\text{C}$  (3 h) and  $550^\circ\text{C}$  (5 h) in order to decompose the precursor salts to oxides (heating ramps  $1 \text{ K min}^{-1}$ ). To produce a more complete filling of the starting silica spheres, the impregnation step was repeated once for both oxides. In the preparation of the  $\text{Mn}_2\text{O}_3$  particles, 1 g of the silica particles was mixed with 10 mL of  $0.8 \text{ M Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and the solvent (i.e. ethanol) was allowed to slowly evaporate at  $70^\circ\text{C}$  for 24 h. Subsequently, the sample was heated at  $200^\circ\text{C}$  for 10 h with a heating ramp of  $1 \text{ K min}^{-1}$  to decompose the metal salt. The impregnation/heat treatment steps were repeated twice after which the final crystal structure could be obtained by calcination at  $550^\circ\text{C}$  (5 h). For all oxides, the silica template was removed by etching in  $2 \text{ M NaOH}$  at  $90^\circ\text{C}$  for 48 h (adding fresh  $\text{NaOH}$  after 24 h).

### 2.3.1. PEI-modification of metal oxide particles

PEI-modification by surface polymerization of ethyleneimine was carried out according to a protocol described by Rosenholm and Linden [28]. Prior to the reaction, 1.0 g of silica particles was degassed at  $70^\circ\text{C}$  for more than 6 h to remove residual physisorbed water. Under inert argon atmosphere, anhydrous toluene (15 mL) was added to the silica particles together with catalytic amounts of acetic acid and 0.4 mL of ethyleneimine. The mixture was then refluxed at  $75^\circ\text{C}$  over night. After the polymerization reaction, the sample was washed 3 times with toluene and 3 times with methanol and subsequently dried in a vacuum oven at room temperature. The PEI-modification of the other metal oxides was carried out using a similar protocol. However, the amounts of ethyleneimine were scaled according to the corresponding surface areas of the metal oxides.

### 2.3.2. Aminopropyl-modification of silica particles

1.0 g of silica particles was degassed at  $70^\circ\text{C}$  for more than 6 h to remove residual physisorbed water. Anhydrous toluene (15 mL) was added under inert argon atmosphere to the silica spheres together with 0.4 mL of APTES. The mixture was subsequently refluxed at  $110^\circ\text{C}$  over night. After completed functionalization, the sample was washed 3 times with toluene and dried in a vacuum oven at room temperature. Finally, the modified sample was aged at  $120^\circ\text{C}$  for 1 h.

## 2.4. Characterization

The morphology of the materials was characterized using SEM. It was necessary to sputter the samples with gold before analysis. The surface area, mesopore volume, and mesopore size of the materials were determined by nitrogen physisorption measurements at  $-196^\circ\text{C}$ . The total pore volume was taken at  $0.98 P/P_0$ , while the Barrett–Joyner–Halenda (BJH) model based on the desorption branch of the isotherm, was used in the pore size calculations. TGA was used to determine the degree of functionalization of the different oxide samples and confirmed by DSC. Finally, the electrokinetic properties of the particles were obtained by zeta potential measurements. The pH was adjusted to 2, 7, and, 10 using  $\text{HCl}$  and  $\text{KOH}$ .

## 2.5. Capillary packing

### 2.5.1. Pressure slurry packing

At the beginning of the packing the total length of the capillaries was 45 cm for CLC and 50 cm for CEC. The outlet of the capillary was fixed together with a mechanical frit and the capillary was packed

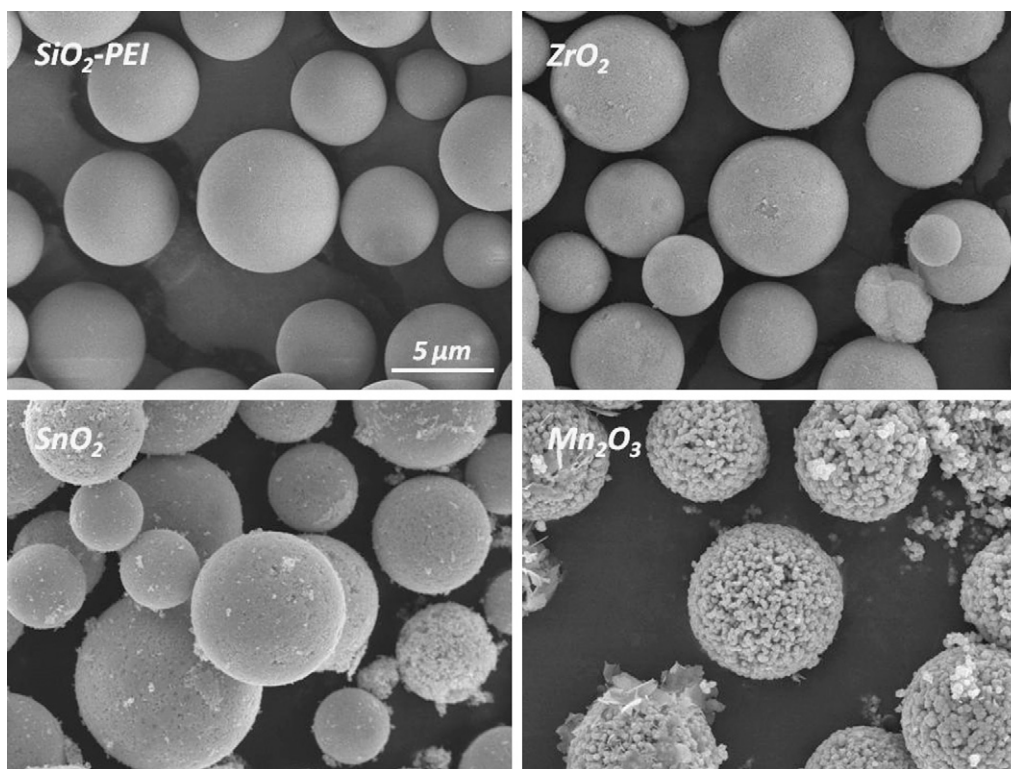
at 400 bars pressure with ODS material in methanol for a length of approximately 5 cm keeping the slurry in a pre-column with dimensions of  $4.6 \text{ mm} \times 50 \text{ mm}$ , with a volume of about 0.830 mL. The ODS material was compressed until it was tightly packed (the whole procedure was constantly followed using a microscope). The solvent was changed into water and water was pumped through the capillary for approximately 20–30 min. After that the ODS material was sintered into a frit with a laboratory-made electrical heated wire by simply heating the capillary at about  $700^\circ\text{C}$  for 7–8 s, while keeping the high pressure on. The ODS material was not properly sintered if the capillary was filled with methanol during the heating step. The mechanical frit was removed and the extra ODS material was removed by flushing at 150 bar with methanol. After this the capillary was packed with the packing material using slurry of approximately 15 mg of packing material in methanol (1 mL). After packing the capillary up to a length of 23 cm, the slurry was changed into pure methanol and the material was compressed. The slurry was kept in constant motion to avoid sedimentation of the particles by turning the packing column up and down or by hitting it to make it vibrate. When no more particles were coming into the capillary, the packing column was opened, the slurry was vortexed and the pressure was applied again. When the capillary was packed with enough packing material, the material was compressed. Then the second frit was made by packing with ODS (in methanol), and after flushing with water for 20 min, the ODS material was sintered in a similar way as the first frit. The extra material was removed with methanol. There was no compression after ODS packing in order not to mix the ODS with the packing material. To note is that the frit material was different from the packing material, which could affect the zones of the analytes (moving from the unpacked zone through the frit to the packed segment). The UV window was burnt 1.5 cm after the second frit and the capillary was cut to its final length (end of the capillary 8.5 and 6.5 cm after the UV-window for CEC and CLC, respectively). Two different kinds of capillaries were prepared: one packed over 25 cm (fully packed) and one packed over 10 cm (short-packed).

### 2.5.2. Electrokinetic packing

In this process the packing of the capillary was done with the help of the EOF. The first frit was made in the same way as in the slurry packing procedure and the capillary was flushed with a small volume of BGE (phosphate buffer, pH 2.0,  $I = 20 \text{ mM}$ ). After that the end of the capillary opposite to the frit was dipped into the slurry (particles in some electrolyte) and the other end was dipped into the BGE solution. During this step a pressure of 7 bars and a voltage of +5 kV were applied to the inlet vial for 3 min. This step was repeated 5 times, and between each repetition the slurry was vortexed. In a second step, both ends of the capillaries were dipped into the BGE and a positive voltage (5 kV) was applied for 15 min. During these two steps the particles were transferred into the capillary and finally compressed. In the capillary the particles were compressed with high pressure and the second frit was made with the LC pump with the same process as described for the packing with pressure.

## 2.6. Capillary electrochromatography

For CEC studies, phosphate and Tris buffers at pH 7.0 with total ionic strengths of 10 mM were used. BGEs were prepared by mixing appropriate volumes of methanol or acetonitrile (ACN) and aqueous buffer. The pH of the buffer was adjusted using concentrated sodium hydroxide prior to mixing with the organic component. BGEs were filtered ( $0.45 \mu\text{m}$ ) prior to use in CEC. The running voltages varied between  $-5$  and  $-25 \text{ kV}$ , and both BGE vials were pressurized during runs at 7 or 8 bars to prevent the formation of bubbles. Sample injection was made by pressure at 7 bars for 12 s.



**Fig. 1.** SEM image of the PEI-modified SiO<sub>2</sub> spheres as well as unmodified ZrO<sub>2</sub>, SnO<sub>2</sub>, and Mn<sub>2</sub>O<sub>3</sub> spheres (the same scale bar in all images).

The concentrations of the model compounds ranged between 20 and 50 μg mL<sup>-1</sup>. The detection wavelength was 200 nm and all runs were conducted at room temperature.

### 2.7. Capillary liquid chromatography

Mobile phases for LC comprised 10 mM phosphate buffer at pH 7.0 (ionic strength of 20 mM) or 21.5 mM Tris-HCl at pH 7.0 (ionic strength of 20 mM). The eluent flow rate was 600 nL min<sup>-1</sup> and the injection volume was 50–60 nL in the commercial device, and the corresponding values were 590 nL min<sup>-1</sup> and 100 nL in the home-built device. UV-detection was made at 214 nm. The concentrations of the model compounds ranged between 5 and 50 μg mL<sup>-1</sup>. All runs were carried out at room temperature.

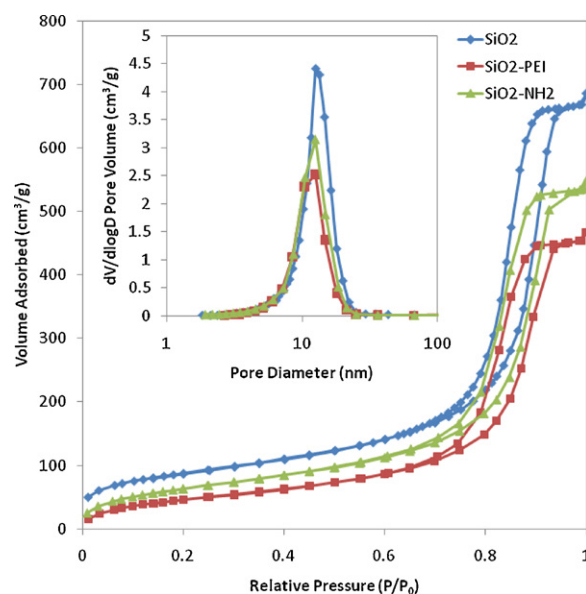
## 3. Results and discussion

### 3.1. Stationary phase materials

The nanocasting method has proven to be useful in the preparation of metal oxide materials with the same morphology as the starting silica template, while forming an inverse replica of the mesopore structure [27,29]. SEM of the micrometer-sized silica spheres together with the different nanocast metal oxide spheres are depicted in Fig. 1. From these images it is evident that the replica sphere sizes remain the same as for the original silica template. However, the replica spheres display a much coarser surface compared to silica (especially Mn<sub>2</sub>O<sub>3</sub>), which is associated with the formation of particle aggregates within the silica matrix during the heating stage. These aggregates give rise to the larger set of mesopores observed in the nitrogen sorption measurements. Surface modification with either ethyleneimine or APTES did not have any visible effect on the morphology (observed by SEM).

Nitrogen physisorption was used to determine the specific surface area, mesopore volume, pore size, and degree of hydropho-

bicity of the surface modified particles, and the results are summarized in Table 1. Fig. 2 shows isotherms of the SiO<sub>2</sub>, PEI-functionalized SiO<sub>2</sub>, and aminopropyl-functionalized SiO<sub>2</sub>, which all have sharp nitrogen uptakes in the relative pressure range 0.8–0.9. From the nitrogen physisorption data it was possible to extract the specific BET surface area (317 m<sup>2</sup> g<sup>-1</sup>), the mesopore volume (1.03 cm<sup>3</sup> g<sup>-1</sup>), and pore size (~12 nm) for the pure silica sample. The lower surface areas and pore volumes for the modified samples due to pore filling and mass increase indicate successful



**Fig. 2.** Nitrogen isotherm of samples SiO<sub>2</sub> (12 nm), PEI-functionalized SiO<sub>2</sub>, and aminopropyl-modified SiO<sub>2</sub> measured at 77 K. (Inset) The derived pore size distribution plots (BJH model based on the desorption branch).

**Table 1**  
Summary of the textural properties obtained from nitrogen physisorption.

Sample	BET surface area [m <sup>2</sup> g <sup>-1</sup> ]	Pore volume [cm <sup>3</sup> g <sup>-1</sup> ]	BJH <sub>des</sub> pore diameter [nm]	c value (N <sub>2</sub> )
SiO <sub>2</sub>	317	1.03	12.0	117
SiO <sub>2</sub> -PEI	180	0.70	10.9	31.3
Aminopropyl-modified SiO <sub>2</sub>	240	0.82	10.8	43.8
ZrO <sub>2</sub>	159	0.49	6.2; 33	68.7
SnO <sub>2</sub>	63	0.18	4.7; 35	64.8
Mn <sub>2</sub> O <sub>3</sub>	58	0.18	10.9	96.6
ZrO <sub>2</sub> -PEI	132	0.53	6.2; 33	28.8
SnO <sub>2</sub> -PEI	60	0.16	5.0; 35	16.9
Mn <sub>2</sub> O <sub>3</sub> -PEI	37	0.13	10.5	45.5

functionalization of the silica spheres. However, the surface areas and pore volumes were still very high for effective use in LC applications. This is corroborated by only a slight decrease of the mean pore diameter (~1 nm) in these samples. The corresponding surface area and pore volume values for the ZrO<sub>2</sub>, SnO<sub>2</sub>, and Mn<sub>2</sub>O<sub>3</sub> particles were typically lower. However, one has to keep in mind that the skeletal densities of these oxides are 2–3 times higher than that of amorphous silica. The metal oxide spheres contain two types of mesopores; the smaller-sized set of pores is the result of silica templating where the silica pore walls become pores in the replica, while the larger pores are from voids between the particle aggregates as discussed above.

The *c* values obtained from the BET plot were used to estimate the degree of PEI- and NH<sub>2</sub>-functionalization. The *c* value of non-functionalized silica was 117 and the value was assumed to decrease after the modification step (more hydrophobic). The value was 31.3 for the PEI-modified sample, while it was 43.8 for the aminopropyl-functionalized SiO<sub>2</sub> sample. The existence of microporosity in the non-modified silica sample can also contribute to a high *c* value. However, for the modified samples no sign of microporosity was detected.

TGA was used to determine the degree of surface modification (summarized in Table 2). PEI was decomposed in several steps over the temperature range 180–700 °C on the modified SiO<sub>2</sub> sample, indicating different types of binding strengths of the PEI moieties. This is corroborated by the exothermic peaks in the DSC plot. The surface excess values for the different PEI-modified metal oxide materials were all in the range of 7–13 μmol m<sup>-2</sup>. The large values indicate that the PEI dendrimers are stacked in several layers. On the contrary, the aminopropyl-modified SiO<sub>2</sub> sample only had a surface excess of 2.9 μmol, which is close to the values reported in the literature for a monolayer coverage of SBA-15-type silica [28]. Thus, we can conclude that the density of amino groups on the PEI-modified samples was higher than that on aminopropyl-modified SiO<sub>2</sub>.

The higher density of amino groups on the PEI materials should also be observed in zeta potential measurements. The zeta potential was measured at three different pH values (2, 7, and 10) and

**Table 2**  
Summary of the TGA and zeta potential measurements for the surface modified silica and metal oxide samples (range 180–700 °C).

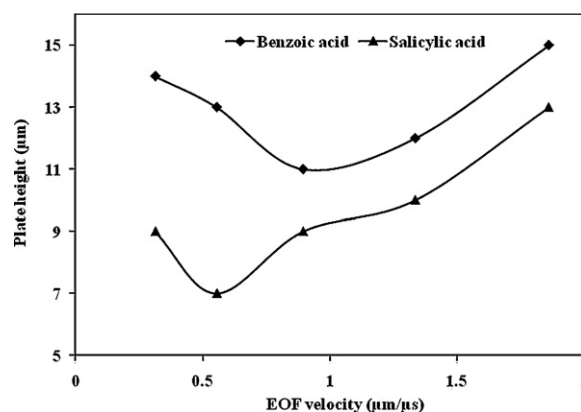
Sample	Organic groups (mmol g <sup>-1</sup> )	Surface excess (μmol m <sup>-2</sup> )	Zeta potential (mV)		
			pH 2	pH 7	pH 10
SiO <sub>2</sub>			16.2	-51.9	-62.3
SiO <sub>2</sub> -PEI	4.10	12.9	55.9	45.0	0.67
Aminopropyl-modified SiO <sub>2</sub>	0.93 <sup>a</sup>	2.93 <sup>a</sup>	24.4	-8.2	-71.5
ZrO <sub>2</sub> -PEI	1.48	9.33	21.0	36.8	-8.64
SnO <sub>2</sub> -PEI	0.44	7.02	25.3	22.4	-55.4
Mn <sub>2</sub> O <sub>3</sub> -PEI	0.65	11.7	33.1	-24.7	-28.2

<sup>a</sup> Only the combustible part of APTES is taken into account in the calculations.

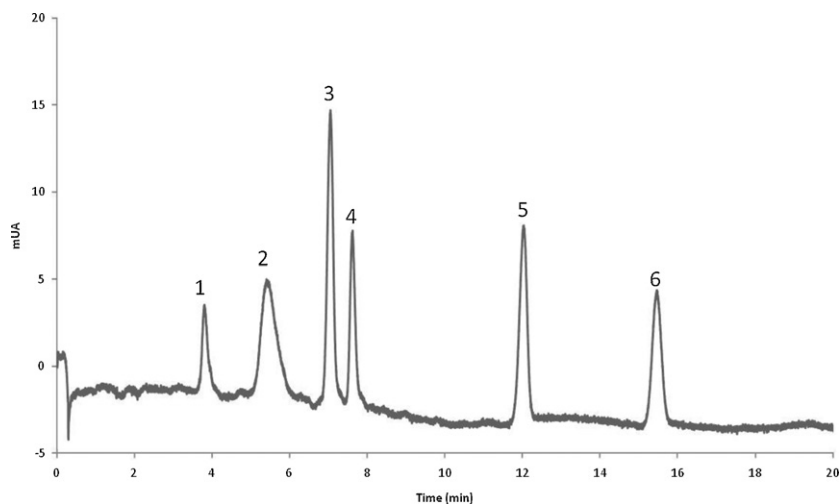
the results are summarized in Table 2. The starting silica sample had clearly a negative zeta potential (close to -50 mV) at neutral pH and an isoelectric point (IEP) at around pH 3. The amino-functionalizations were applied to shift the isoelectric point (IEP) to higher pH values. However, for the aminosilane-modified silica sample, the zeta potential was still negative at pH 7, although much lower (-8 mV) than for the bare silica particles. What is interesting is that the PEI-modified silica sample had a slightly positive zeta potential even at pH 10 and was clearly positive at pH 7 (+45 mV). These values are close to the ones reported for similar surface modifications on mesoporous silica [24]. Water stability tests indicated that the PEI-functionalization of the SiO<sub>2</sub>-PEI sample was chemically stable under the conditions used in this study. Similar shifts in the IEPs were achieved for the ZrO<sub>2</sub>, SnO<sub>2</sub>, and Mn<sub>2</sub>O<sub>3</sub> samples functionalized with PEI, although not to the same extent as for silica (Table 2). The zeta potential values for ZrO<sub>2</sub>-PEI and SnO<sub>2</sub>-PEI are clearly positive at pH 7, while the Mn<sub>2</sub>O<sub>3</sub>-PEI sample is negative. The lower zeta potential values could be due to the slightly lower surface coverage for these metal oxides compared to the SiO<sub>2</sub>-PEI sample. Another possible explanation is the lower chemical stability of the anchoring points of the PEI dendrimers to these oxides at high pH.

### 3.2. Packing procedures

The packing of the capillary was easily done with PEI-functionalized SiO<sub>2</sub> and aminopropyl-functionalized SiO<sub>2</sub> (approx. 23 cm in around 1 h) even though it was necessary to vortex the slurry every 10 or 15 min. The slurry was made in methanol. Water was also tested but the packing process was much slower than with methanol. The effect of ultrasonic energy on the slurry forma-



**Fig. 3.** Plate heights of benzoic acid and salicylic acid as a function of EOF velocity in capillaries packed with PEI-functionalized SiO<sub>2</sub>. Capillary: 100 μm I.D., 23.0/33.0 cm (packed/total), effective length 24.5 cm; BGE: aqueous phosphate buffer at pH 7.0 (ionic strength of 10 mM); voltages from -5 to -25 kV; temperature 25 °C; injection at 7 bar for 12 s; both ends of the capillary was pressurized with 7 bar; detection at 200 nm.

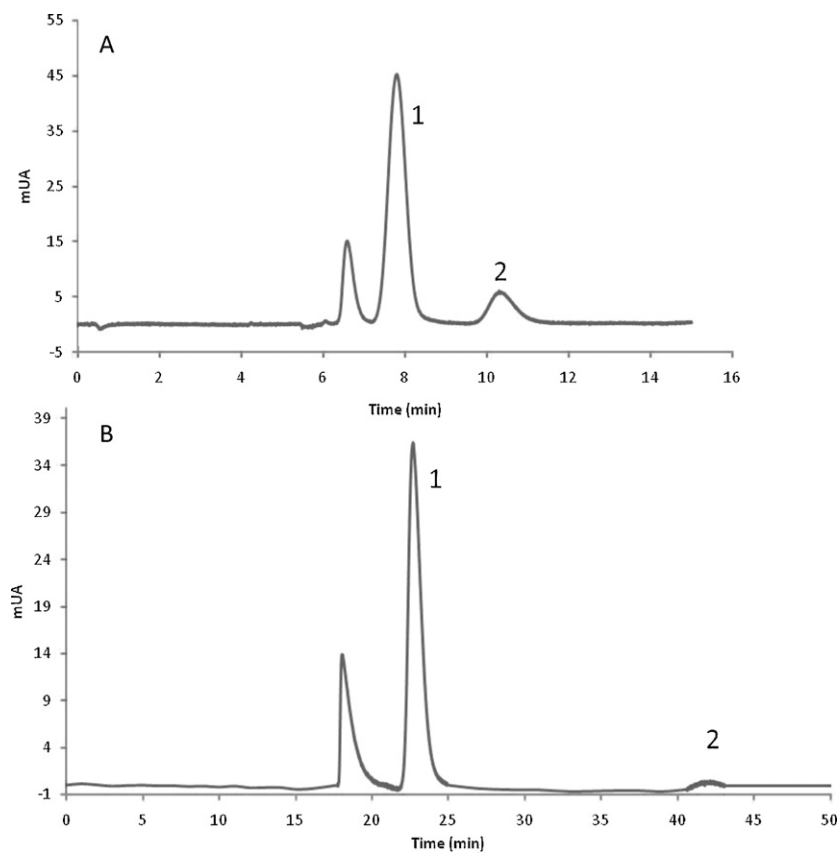


**Fig. 4.** CEC separation of (1) acetone, (2) benzoic acid, (3) 2,6-dihydroxybenzoic acid, (4) 2,5-dihydroxybenzoic acid, (5) 2,4-dihydroxybenzoic acid and (6) 3,5-dihydroxybenzoic acid (analyte concentrations were  $50 \mu\text{g mL}^{-1}$ ); EOF, acetone peak; capillary 23.0/33.0 (packed/total), effective length  $24.5 \text{ cm} \times 100 \mu\text{m}$  I.D. pressure slurry packed with PEI-functionalized  $\text{SiO}_2$ ; BGE: methanol in phosphate buffer (pH 7.0 and  $I=20 \text{ mM}$ ) 80/20% (v/v); voltage:  $-30 \text{ kV}$ ; temperature:  $25^\circ\text{C}$ ; pressure (both sides): 7 bars; injection: 7 bars during 12 s; detection wavelength: 200 nm.

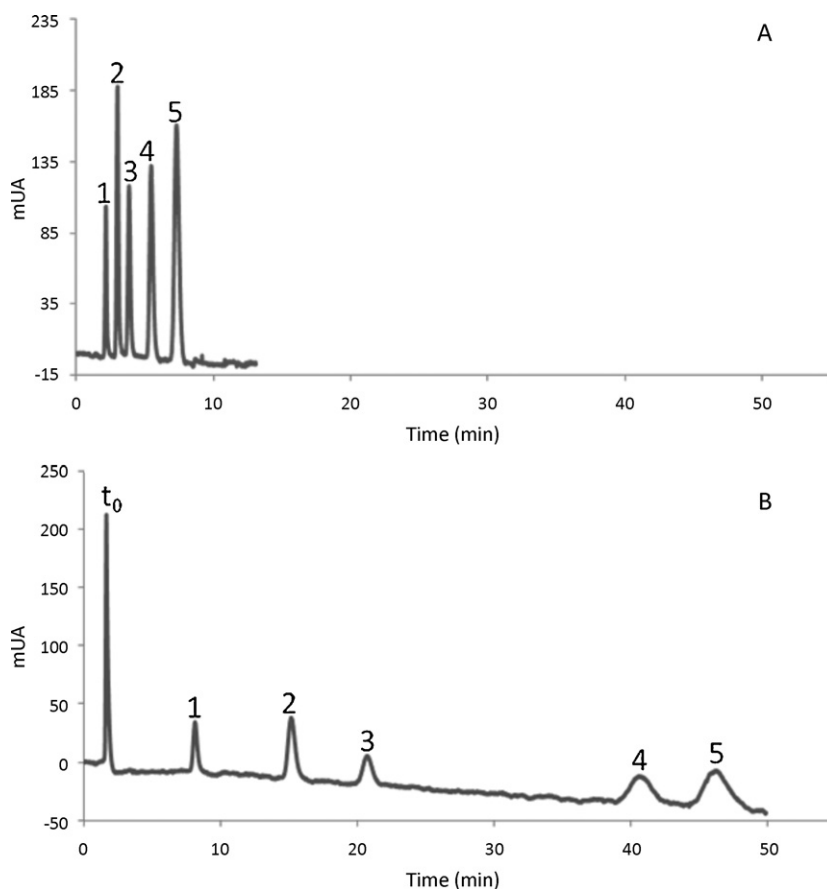
tion and on the particle sizes was studied by SEM after the packing procedure and the ultrasound decreased the particle sizes. No differences were found in the case of the columns packed without ultrasound. The effect of pressure on the properties of the particles was studied after 40 runs. No significant differences were found between the original and the packed material. Therefore,

we dare to claim that pressure did not affect the materials under study.

PEI-functionalized  $\text{SnO}_2$ ,  $\text{ZrO}_2$ , or  $\text{Mn}_2\text{O}_3$  particles packed under the same slurry packing conditions caused blockages in the capillary, resulting in successful packing of only a few centimeters of the capillary (3 or 4 cm). Through the microscope it was observed that



**Fig. 5.** CEC separation of (1) phenol and (2) benzene (analyte concentrations  $50 \mu\text{g mL}^{-1}$ ); EOF acetone peak: (A) PEI-functionalized  $\text{SiO}_2$ ; 23.0/33.0 (packed/total), effective length  $24.5 \text{ cm} \times 100 \mu\text{m}$  I.D. pressure slurry packed; BGE: phosphate buffer (pH 7.0 and  $I=20 \text{ mM}$ ); voltage:  $-10 \text{ kV}$ ; temperature:  $25^\circ\text{C}$ ; pressure (both sides): 7 bars; injection: 7 bars during 12 s; detection wavelength: 200 nm. (B) Aminopropyl-functionalized  $\text{SiO}_2$ ; capillary 33.0/23.0 (total/packed), effective length  $24.5 \text{ cm} \times 100 \mu\text{m}$  I.D. pressure slurry packed; BGE: phosphate buffer (pH 7.0 and  $I=10 \text{ mM}$ ); voltage:  $-5 \text{ kV}$ ; temperature:  $25^\circ\text{C}$ ; pressure (both sides): 7 bars; injection: 8 bars during 12 s; detection wavelength: 200 nm.



**Fig. 6.** Effect of type of buffer at constant ionic strength on the separation of benzoic acid and its derivatives by CLC using PEI-functionalized  $\text{SiO}_2$ . Mobile phase: (A) sodium phosphate pH 7 ( $c = 10 \text{ mM}$ ,  $I = 20 \text{ mM}$ ) and (B) Tris-HCl pH 7 ( $c = 21.5 \text{ mM}$ ,  $I = 20 \text{ mM}$ ). Length of capillary; 15/22 cm (packed/total) ( $100 \mu\text{m}$  I.D.). Flow rate:  $590 \text{ nL min}^{-1}$  (flow pump  $0.15 \text{ mL min}^{-1}$ );  $p_{\text{col}} = 10.7 \text{ MPa}$ ; inj.:  $\sim 100 \text{ nL}$  (time split); sample concentration:  $\sim 5\text{--}10 \mu\text{g mL}^{-1}$  in  $\text{H}_2\text{O}$ , UV: 214 nm. Numbering of compounds: ( $t_0$ ) methanol; (1) benzoic acid; (2) 2,6-dihydroxybenzoic acid; (3) 2,5-dihydroxybenzoic acid; (4) 2,4-dihydroxybenzoic acid; (5) 3,5-dihydroxybenzoic acid.

the positively charged particles were adsorbed onto the negatively charged capillary, and finally the capillary was totally blocked, hindering further packing. In order to solve this adsorption problem two different capillary coatings, APTES with positively charged amino groups and close to neutral polyacrylamide, were tested. Even though the PEI-functionalized  $\text{ZrO}_2$  material could be successfully packed into the capillary, these capillaries could not be used in CEC because of the constant formation of bubbles, which disconnected the electric field (unstable current). With the polyacrylamide coating the capillary could be packed with  $\text{SnO}_2$ -PEI particles, but only to a length of 10 cm. Most probably the acrylamide coating could not withstand methanol used for packing. In addition, a black polymerized material was observed inside the capillary.

Pre-conditioning the capillary with a pH 2.0 solution was useful when capillaries were packed with PEI-functionalized  $\text{Mn}_2\text{O}_3$  particles, opposite to PEI-functionalized  $\text{ZrO}_2$  particles that were blocked already after a few centimeter of packing.

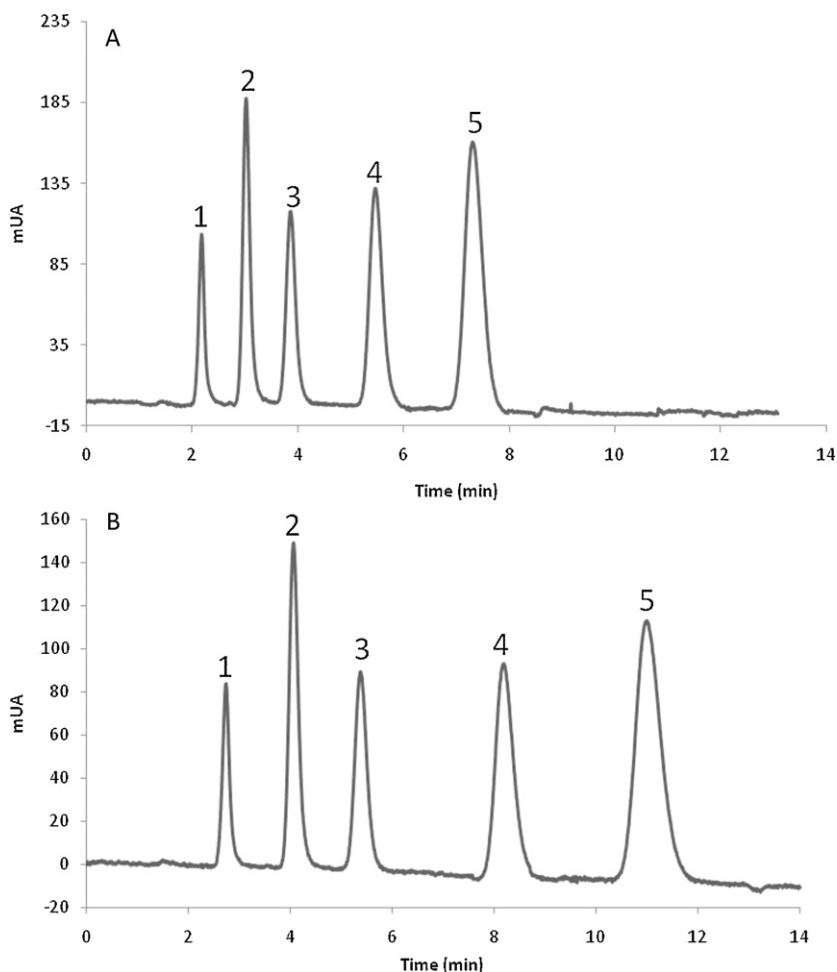
The PEI-functionalized  $\text{SiO}_2$  particles could be packed with the electrokinetic method but this method was more time-consuming as the slurry had to be vortexed every 3 min. Taking into account that the main force that pushed particles into the capillary was the EOF, it was necessary to apply pressure at the inlet to avoid bubble formation and thus to achieve a stable current. The electrokinetic packing was done at pH 2.0 to avoid dissociation of the silanol groups on the silica capillary surface and thus to minimize interactions between the negative charges of the capillary and the positively charged particles. Unfortunately, only 3 cm of the capillary could be packed with this method until

bubbles started to appear around the frit. In addition, because some of the metal oxides are slightly electrically conductive ( $\text{SnO}_2$  and  $\text{Mn}_2\text{O}_3$ ) the electrokinetic packing procedure could not be used with these materials (formation of bubbles due to redox reactions). Neither could the PEI-functionalized  $\text{ZrO}_2$  material be packed with the electrokinetic packing procedure. Accordingly, further studies were carried out only with PEI-functionalized  $\text{SiO}_2$ .

### 3.3. Influence of pH and voltage on EOF in capillaries packed with PEI-functionalized $\text{SiO}_2$

PEI-functionalized  $\text{SiO}_2$  packed capillaries (pressure slurry packed) were studied by CEC. The EOF was investigated at pH range 2.2–7.7 (pH 2.2, 3.5, 6.0, 7.0, and 7.7) with phosphate buffers (number of repetitions at each pH value was six but in the case of pH 2 only two repetitions were made). At all the pH values studied the EOF was negative because of the positively charged PEI groups. The EOF remained rather stable between pH 6 and 7.7 ( $\mu_{\text{eo}}$  about  $-2.1 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ ). The slower EOF at basic pH values compared to that at pH 2 ( $\mu_{\text{eo}}$  about  $-2.7 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) evidenced a decreased surface charge on the particles with an increase in the pH value.

The capillary packed with PEI-functionalized  $\text{SiO}_2$  could well withstand voltages from  $-5$  to  $-25 \text{ kV}$ . For this investigation the total length of the capillary was 33.0 cm, the length to the detector was 24.5 cm, and the length of the packed capillary was 23.0 cm. With a 10 mM phosphate buffer at pH 7 the corresponding currents increased linearly ( $R^2 = 0.9778$ ) from  $-2.5$  and  $-23.0 \mu\text{A}$ . The



**Fig. 7.** CLC of benzoic acid and its derivatives with a capillary packed with PEI-functionalized SiO<sub>2</sub>. (A) 10 mM sodium phosphate pH 7 ( $I = 20$  mM) and (B) 5 mM sodium phosphate pH 7 ( $I = 10$  mM). Cap. 15/22 cm (packed/total) (100  $\mu$ m I.D.). Flow rate: 590 nL min<sup>-1</sup>; Inj:  $\sim$ 100 nL (time split), UV: 214 nm. Numbering of compounds: (1) benzoic acid; (2) 2,6-dihydroxybenzoic acid; (3) 2,5-dihydroxybenzoic acid; (4) 2,4-dihydroxybenzoic acid; (5) 3,5-dihydroxybenzoic acid.

EOF increased linearly from  $-10$  to  $-25$  kV with a correlation factor of  $R^2 = 0.9784$ . However, at  $-5$  kV the EOF was much faster than expected (no reasonable explanation can be given at this stage). A mixture of benzoic acid and salicylic acid, with acetone as the EOF marker, was tested at the voltage range mentioned above. The repeatabilities of the migration times (RSD;  $n = 6$ ) were 2.3–2.5% at  $-5$  kV, 0.5–2.8% at  $-10$  kV, 1.0–2.2% at  $-15$  kV, 0.7–0.8% at  $-20$  kV, and 3.1–5.5% at  $-25$  kV.

The efficiencies were between 25 000 and 47 000 plates for the last migrating compound (salicylic acid), calculated from the half widths of the compound peaks. The van Deemter curves of benzoic acid and salicylic acid seen in Fig. 3 shows that the lowest plate heights were obtained at  $0.9 \mu\text{m} \mu\text{s}^{-1}$  for benzoic acid and at  $0.6 \mu\text{m} \mu\text{s}^{-1}$  for salicylic acid.

#### 3.4. Separation of acidic and neutral compounds by CEC using capillaries packed with functionalized SiO<sub>2</sub>

The applicability of the PEI-functionalized SiO<sub>2</sub> packed capillaries (pressure slurry packed) to the separation of analytes by CEC was tested using a mixture of four isomers of dihydroxybenzoic acid and two organic acids. Electropherograms of repeated injections of the acids demonstrated repeatable results with migration time relative standard deviations between 0.9 and 5.4% for all investigated compounds. The influence of organic modifier (methanol and acetonitrile) was investigated, and 20% (v/v) methanol in the phos-

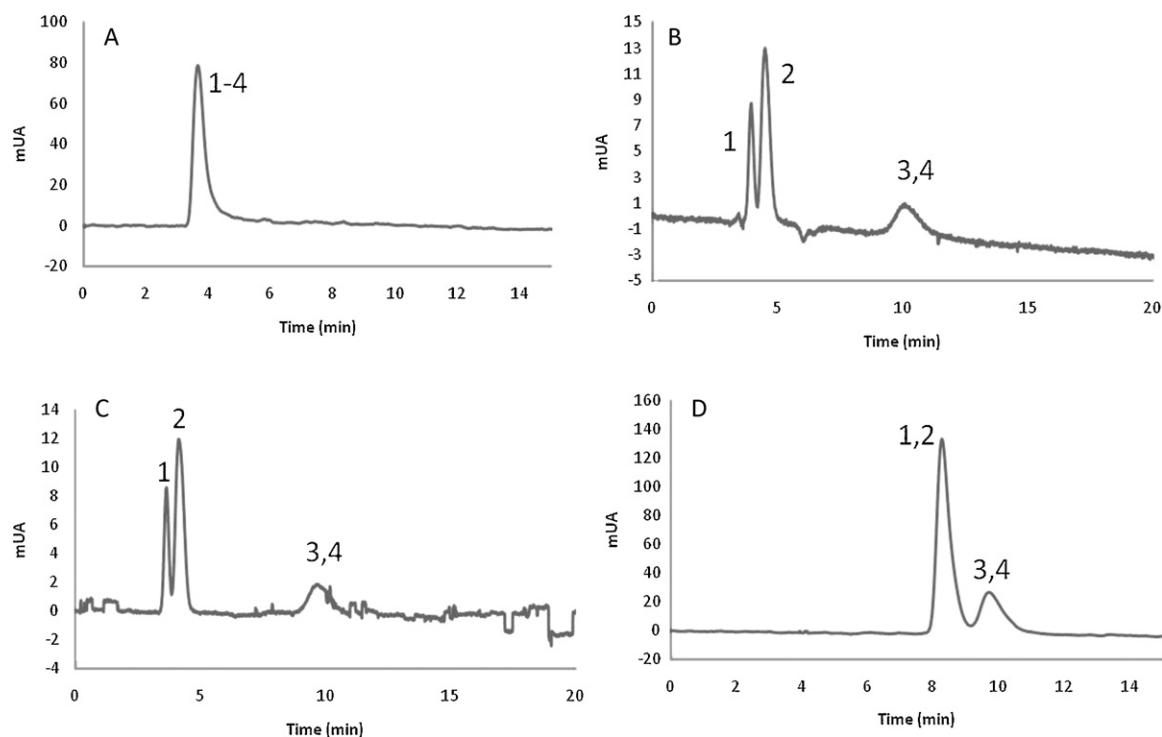
phate BGE solution shortened the total analysis time by affecting the viscosity from almost 30 min to 16 min (Fig. 4).

The separation of some neutral compounds was carried out to investigate the hydrophobicity of the PEI-functionalized SiO<sub>2</sub> material. A mixture of acetone, phenol, and benzene proved the material to be hydrophobic (Fig. 5a). Substituting the relatively long PEI dendrimer with a shorter aminopropyl group (R<sub>2</sub>SiO(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub>) proved that even aminopropyl-modified SiO<sub>2</sub> resulted in, not only electrostatic interactions, but also hydrophobic interactions with the analyzed compounds. Separation of the neutral analytes (see above) on an aminopropyl-functionalized SiO<sub>2</sub> packed capillary is shown in Fig. 5b. However, higher voltages than  $-5$  kV could not be used without having bubbles without breaking the electric field. Accordingly, in the aminopropyl-modified SiO<sub>2</sub> packed capillary the EOF was very low, resulting in a very long analysis time and broad peaks. The retention factor of benzene on PEI-functionalized SiO<sub>2</sub> was 0.56–0.62 and that on aminopropyl-modified SiO<sub>2</sub> 0.81–1.32. This is in contradiction with the BET data ( $c$  values, Table 1), demonstrating that on these materials the retention of benzene is not solely due to hydrophobic interactions.

#### 3.5. Application of functionalized metal oxide particles for CLC

The applicability of PEI-functionalized SiO<sub>2</sub> particles as packing materials in CLC was also studied. Under the same conditions





**Fig. 8.** CLC substituted benzoic acids with capillaries (100  $\mu\text{m}$  I.D.) pressure slurry packed with: (A) PEI-functionalized  $\text{Mn}_2\text{O}_3$  (packed at pH 2.0). Mobile phase: 10 mM sodium phosphate, pH 7.0 ( $I=20$  mM). Capillary 20.5/28 cm (packed/total) (100  $\mu\text{m}$  I.D.). Flow rate: 600  $\text{nL min}^{-1}$ ; Inj: 200  $\text{nL}$ ; UV: 214 nm; Co-elution of the analytes: (1) 2,6-dihydroxybenzoic acid; (2) 2,5-dihydroxybenzoic acid; (3) 2,4-dihydroxybenzoic acid; (4) 3,5-dihydroxybenzoic acid. The sample concentrations were 100  $\mu\text{g mL}^{-1}$ . (B) PEI-functionalized  $\text{SnO}_2$ , PAA-coated capillary 7.5/17 cm (packed/total); Mobile phase, analytes, and running conditions as in A. (C) PEI-functionalized  $\text{SnO}_2$ , PAA-coated capillary 7.5/17 cm (packed/total); Mobile phase: 15 mM sodium phosphate, pH 7.0 ( $I=30$  mM); Analytes and running conditions as in (A). (D) PEI-functionalized  $\text{SnO}_2$ , Uncoated fused silica capillary 11.5/20 cm (packed/total). Mobile phase, analytes, and running conditions as in (A).

(ionic strength of 20 mM and pH 7) the retention times of acidic benzoic acid derivatives were much shorter when sodium phosphate was used as buffer compared to Tris-HCl, as seen from Fig. 6. The effect of ionic strength of sodium phosphate resulted, as expected, in shorter retention times at the higher the ionic strength, proving the existence of ion-exchange mechanism (Fig. 7). The PEI-functionalized  $\text{Mn}_2\text{O}_3$  material, that was not suitable in CEC due to instable current and the formation of bubbles could be used as stationary phase in CLC, but unfortunately all analytes coeluted at 4 min (Fig. 8A). A capillary packed with PEI-functionalized  $\text{SnO}_2$  provided only three peaks in the chromatogram (Fig. 8B and C). The ionic strength affected only slightly the separation of the model analytes compared to that carried out in capillaries packed with PEI-functionalized  $\text{SiO}_2$  particles. When the capillary surface was covalently modified with polyacrylamide before the packing (Fig. 8C and D) compounds 3 and 4 were overlapped and eluted later in both PEI-functionalized  $\text{SnO}_2$  packed capillary columns. However, the retention times of the earlier eluting compounds 1 and 2 were much shorter and the resolution was better in the PAA-coated packed capillary (Fig. 8C). The results prove that the surface area of the packing material played more important role than the length of the column in the separation.

The effect of the length of the carbon chain in the functionalized materials was studied by comparing  $\text{SiO}_2$ -PEI and aminopropyl-modified  $\text{SiO}_2$  materials by CLC. Aromatic acids, with pKa values ranging from 1.30 to 4.19, were used as model compounds. At pH 3 where all the compounds were not yet fully dissociated the model compounds interacted strongly with both stationary phase materials demonstrating ion exchange and hydrophobic interactions. At pH 7 all the compounds were fully dissociated but baseline separation of the model compounds was only achieved with  $\text{SiO}_2$ -PEI packed material, which contains longer carbon chain than the aminopropyl-modified  $\text{SiO}_2$  material.

#### 4. Conclusions

Novel PEI-functionalized metal oxide 5  $\mu\text{m}$  particles were prepared and used for the fabrication of capillary columns for CEC and CLC. The materials prepared by nanocasting included PEI-functionalized  $\text{SiO}_2$ ,  $\text{ZrO}_2$ ,  $\text{Mn}_2\text{O}_3$ , and  $\text{SnO}_2$  particles, as well as aminopropyl-functionalized  $\text{SiO}_2$  particles. The charged materials had both ionic and hydrophobic interactions with the investigated compounds. Different capillary packing procedures were tested. Slurry pressure packing was most practical with PEI-functionalized  $\text{SiO}_2$  and aminopropyl-modified  $\text{SiO}_2$  particles, and the electrokinetic packing method was also useful for the packing with PEI-functionalized  $\text{SiO}_2$  particles. However, the electrokinetically packed capillaries could only be used at very low electric field strengths.  $\text{ZrO}_2$ -PEI particles were successfully slurry packed also into a positively charged APTES-coated capillary and  $\text{SnO}_2$ -PEI particles into a neutral PAA-coated capillary. Pre-conditioning of the capillary with a pH 2.0 electrolyte solution was needed before packing with  $\text{Mn}_2\text{O}_3$ -PEI particles. Among the functionalized metal oxide particles prepared PEI-functionalized  $\text{SiO}_2$  that provided good stability, hydrophobic and ion exchange interactions over a wide pH range proved to be the most useful packing material in both CEC and CLC.

#### Acknowledgments

Financial support was provided by the Research Council for Natural Sciences and Engineering, the Academy of Finland under grants 116288 (M.-L.R.), 127919 (J.-H.S., M.S.) and Magnus Ehrnrooth Foundation (C.B.-P., G.D., M.K., and M.-L.R.). MSc Alain Duchanoy is gratefully acknowledged for the aziridine synthesis and Dr Mikael Järn for conducting the SEM measurements.

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