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Review

Synthesis of spherical porous silicas in the micron and submicron size range: challenges and opportunities for miniaturized high-resolution chromatographic and electrokinetic separations☆

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Abstract

Classical silica technology has reached its limit with respect to an ultimate minimum particle size of about 2 μm in diameter. Here, a novel process is presented which allows one to synthesize porous silica beads and control their particle diameter in situ, within the range of 0.2–2.0 μm. As a result, no sizing is required and losses of silica are avoided. Furthermore, the process enables one to control in situ the pore structural parameters and the surface chemistry of the silica beads. Even though surface funtionalized silicas made according to this process can principally be applied in fast HPLC the column pressure drop will be high even for short columns. In addition, the column efficiency, expressed in terms of the theoretical plate height is about $H \sim 2d_p$ in the best case and limited by the *A* and *C* term of the Van Deemter equation. In other words the gain in total plate number when using 1–2 μm silica beads in short columns is minimal as compared to longer columns packed with 5 μm particles. Capillary electrochromatography (CEC) as a hybrid method enables the application of micron size as well as submicron size particles. This consequently enhances column efficiency by a factor of 5–10 when compared to HPLC. The use of short CEC columns packed with submicron size silicas provides the basis for fast and efficient miniaturized systems. The most significant feature of CEC as compared to HPLC is that the former allows one to resolve polar and ionic analytes in a single run. An alternative method for miniaturization is capillary electrophoresis (CE) which generates extremely high efficiencies combined with fast analysis. Its application, however, is limited to ionic substances.

Keywords: Reviews; Silica; Stationary phases, LC; Porous particles

Contents

☆Dedicated to Professor B.L. Karger, Northeastern University, Boston, MA, USA, on his 60th birthday.

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1. Introduction

About 30 years ago classical column liquid chromatography (CLC) merged into its modern variant high-performance liquid chromatography (HPLC). Comprehensive treatment of the chromatographic processes gave clear directives as to how a highly efficient HPLC system should be designed and operated. With respect to column packings the requirements were to employ microparticulate and mechanically stable supports of 5–10 μm average particle size and to pack these particles in a stainless steel column to a dense and stable bed being held by porous frits. A small pulse of sample was injected on top of the column and separations were performed with a linear flow velocity of 1–5 mm/s.

During this period the major source of silica packings for HPLC were mainly technical-grade silica xerogels of large particle size. They were subjected to consecutive milling and sizing processes to obtain 5–10-μm fractions with acceptable size distributions. For sizing microparticulate silicas novel sizing technology was developed by Alpine (Augsburg, Germany), based on air elutriation. To pack the material into columns, suspensions of these fine particles were filtered through stainless steel columns of 4 and 4.6 mm inner diameter, terminated by a porous frit, at high flow-rates of about 10 ml/min and pressures of up to 600 bar. As the particles were irregular in shape with fines adhering to the surface as a result of the milling process, the columns often showed a larger pressure drop than expected and a poor column longevity due to rearrangement of the particles in the packed bed during separation. As a consequence emphasis was placed on the development of processes by which spherical silica particles could be obtained without milling. However, sizing was still required to narrow the particle size distribution with some exceptions [1,2]. The major siliceous precursors were sodium silicate solutions and stabilized silica sols. Beading was achieved by agglutination of silica sols in the presence of a monomer [1,2] by converting droplets of silica sols into silica hydrogel beads in an immiscible two-phase system [3,4] and by spray drying silica sols [5,6]. Another route was to partially hydrolyze tetraethoxysilane to a condensed polyethoxysiloxane (PES) and to convert droplets of PES into spherical silica hydrogel beads by stirring in a heterogeneous phase [7,8]. All these processes require meticulous control of the reaction parameters and a highly sophisticated technology.

During the 1980s more refined processes were developed to better control the pore structural parameters, the particle size and to reduce the metal impurities in the bulk and the surface of the silicas (as an example see Ref. [9]). In addition, the parent silicas were subjected to specific post-synthetic treatments under hydrothermal conditions, treatment with acids and dehydroxylation/rehydroxylation cycles to stabilize the surface and to create a more homogeneous surface for consecutive chemical surface modification, for example, silanization [10].

Another feature was to control the residual hydroxyl group concentration of silanized silicas. Several attempts were undertaken such as the use of silanes with protective side chains (sterically protected silicas) [11], the formation of an intermediate hydrophilic layer prior to silanization [12] and the formation of so-called end-capped silicas. The objective was to manufacture reversed-phase silicas suitable for the resolution of basic analytes.

An additional route to adjust the hydrophilic character of the silica surface relative to its hydrophobicity is to prepare porous silica/organic hybrid particles by cohydrolysis and cocondensation of tetraethoxysilane and organoalkoxysilanes [13].

2. Novel concepts in the design and processing of silica packings for HPLC

Novel routes in the design and processing of silica packings should include the following features:

(i) processes which enable one to manufacture beads with controlled particle size and size distribution without any subsequent size fractionation;

(ii) adjustable and reproducible pore structural parameters carried out in situ by control of the reaction parameters;

(iii) defined and reproducible surface chemistry of the silica; and

(iv) reproducible and rugged manufacturing process.

It was during 1980 that we started to reinvestigate so called the Stoeber process. Stoeber et al. developed a procedure to synthesize monodisperse non-porous silica particles of about 1 μm size [14]. Tetraethoxysilane was hydrolyzed and condensed in an ethanolic ammonia solution to form silica hydrogel beads, which were then subjected to calcination. By a controlled growth process of the Stoeber particles we were able to manufacture non-porous particles of up to 3 μm [15]. After chemical modification we applied this non-porous packing in reversed-phase (RPLC), ion-exchange (IEC) and hydrophobic interaction chromatography (HIC) of peptides and proteins [16]. The particles were packed into short columns of 30×4 mm and operated at high flow-rates of up to 5 ml/min with a column back pressure of up to 300 bar. The separations were performed in less than 1 min with optimized HPLC systems [17]. Later Barder and Dubois [18] and Kovats et al. [19], developed similar procedures. Nowadays, the columns are commercially available from Bischoff Chromatography, Leonberg and Eichrom, USA (formerly Micra Scientific, USA).

Parallel to research on non-porous silica packings we dealt with the synthesis of silica-rich zeolites of the MFI structure type. The objective was to understand the role of the structure directing agents (templates) during the nucleation and crystallization of these zeolites; and to synthesize template-free materials with the aim of adjusting the particle size of the crystals, within the range of about 100 nm diameter up to about 300 μm.

MFI zeolites possess a three-dimensional pore system with pore diameters between 0.5 and 0.6 nm, which are too small to be used in HPLC. Hence, these tailor-made materials were exclusively employed as catalysts [20,21].

When Mobil researchers first reported the synthesis of ordered mesoporous silicas such as MCM-41 we concentrated on efforts to utilize this concept to process tailor-made silica packings. The classical synthesis of MCM-41 is carried out with a silicate solution and long chain *n*-alkyl ammonium salts at elevated temperatures of about 100°C [22]. The detergent molecules form micelles with the silicate anion species via a process of cooperative interaction and the latter are attached to the outer surface of the micelles [23]. In the case of MCM-41 these micelles are ordered into hexagonally packed rods that form a so-called mesostructured phase with the micelles in the interior of the rods whose walls consists of amorphous silica. Thus, the detergent builds a supramolecular structure rather than ordering on a molecular scale as seen in the MFI synthesis where the template directs the bulk structure. A unidimensional pore system is created and the average pore diameter can be tuned by the *n*-alkyl chain length of the surfactant employed. The detergent molecules are burned out of the silica leaving a porous material with amorphous silica walls of about 1 nm thickness.

The specific surface area amounts to $>1000\ m^2/g$ and the specific pore volume ranges between 0.8 and 2 ml/g. The material is an agglomerated silica of primary particles of about 50 nm diameter. Due to the high porosity and the agglomerated structure the material does not withstand high pressure and, therefore, cannot be used as a packing material for HPLC. In 1995 we synthesized spherical MCM-41 and MCM-48 material using tetraethoxysilane and long chain *n*-alkyltrimethylammonium bromides or *n*-alkylpyridinium bromides as structure directing agents [24]. It was Tanev and Pinnavaia [25], however, who first employed *n*-alkylamines as templates. Yet this synthesis procedure did not yield particles of spherical morphology. The breakthrough occurred when we carried out the hydrolysis and condensation of tetraethoxysilane with long-chain *n*-alkylamines in aqueous ammonia solutions at a pH of 10 (according to Stoeber) using isopropanol as the co-solvent [26]. The procedure is schematically shown in Fig. 1. The reaction started with a homogeneous solution at room temperature or higher. Neither autoclaves nor stirring were required. The average particle diameter of the particles precipitated could be varied and particles having a nearly monodisperse particle size distribution were yielded (see Fig. 2). The mechanism of formation is certainly not analogous to that of MCM-41, i.e., the material does not exhibit a regular packing of pores (long-range order) as seen in MCM-41. X-ray diffractograms at low 2θ values indicate the presence of one peak instead of four as with the MCM-41 material (see Fig. 3). Control of pore size and porosity was achieved by different methods with respect to the MCM-41 synthesis. The particle size was controlled by the water–TEOS molar ratio in the starting

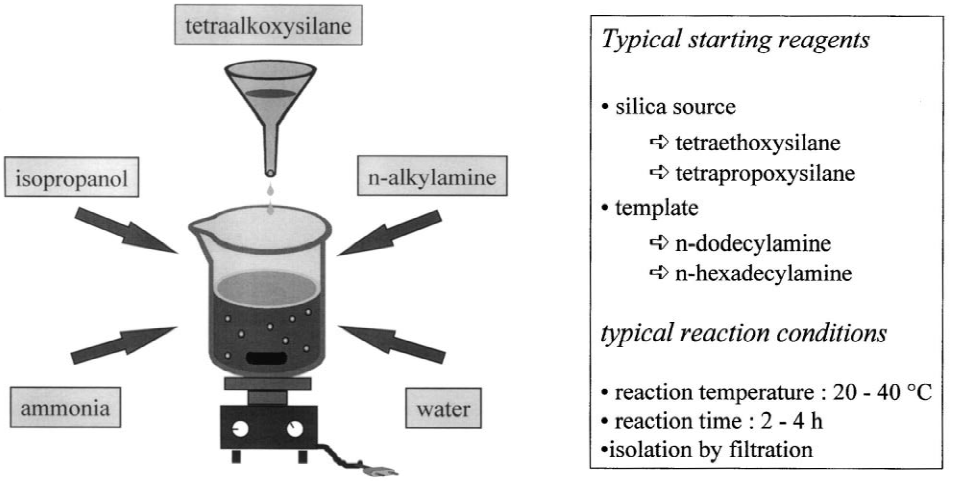


Fig. 1. Synthesis of spherical mesoporous silica.

reaction mixture as well as by the reaction temperature, namely within the range of 15–40°C.

The particular advantage of this method is the stringent control of the particle morphology and particle size which makes sizing into fractions unnecessary. This implies that there is no loss of material due to under and oversizes. The most favourable range of average particle diameter is between 0.2 and 2 μm. Usually the average pore diameter is in the range between 2 and 5 nm. Pore size enlargement is achieved either by a post-synthetic hydrothermal treatment under specific conditions or by using oligomeric and polymeric templates. The silicas obtained are much more hydrothermally stable than amorphous silica xerogels and MCM-41 types of silica. An example is the collapse of long-range order and the pore structure of MCM-41 with hydrothermal treatment. With respect to the surface hydroxyl group concentration the silica products discussed possess an α_{OH} value between 4 and 5 $\mu mol/m^2$, whereas a fully hydroxylated amorphous silica has a α_{OH} value of 8–9 $\mu mol/m^2$ [27] and MCM-41 as a representative of an ordered mesoporous silica has an α_{OH} value of 2–4 $\mu mol/m^2$ [28].

It should be emphasized in this context that monodisperse micron and submicron size polymer beads, e.g., based on polystyrene–divinylbenzene, have been also synthesized.

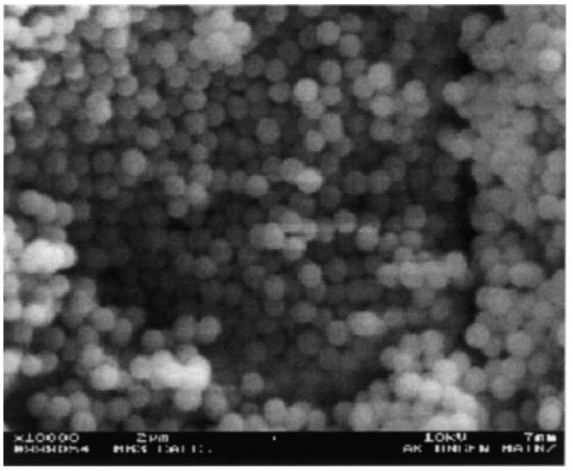
3. The limits of current chromatographic and electrokinetic techniques for fast and high throughput separations

3.1. High-performance liquid chromatography

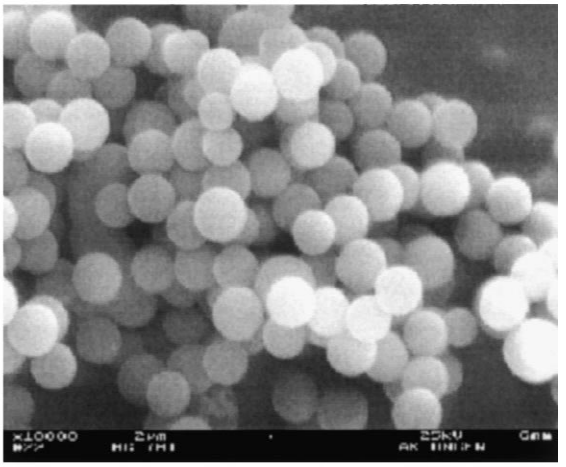
The state-of-the-art in column technology in HPLC was recently reviewed by Rozing [29] and Majors [30]. Typically, columns of 100–250 mm length and 4 and 4.6 mm inner diameter packed with 5-μm reversed-phase silicas are routinely employed. Short columns of about 50 mm in length and 4 and 7 mm I.D. packed with 3–4-μm materials are offered by a number of column manufacturers for fast separations in the order of minutes.

There is, however, a major limitation with respect to the sizing technology of the silica particles that have an average particle diameter of $d_p < 3$ μm. The sizing of narrow fractions of d_p ~1–2 μm by the air elutriation techniques are extremely time consuming, expensive and also less efficient. The same considerations are valid for sedimentation as an alternative sizing technique.

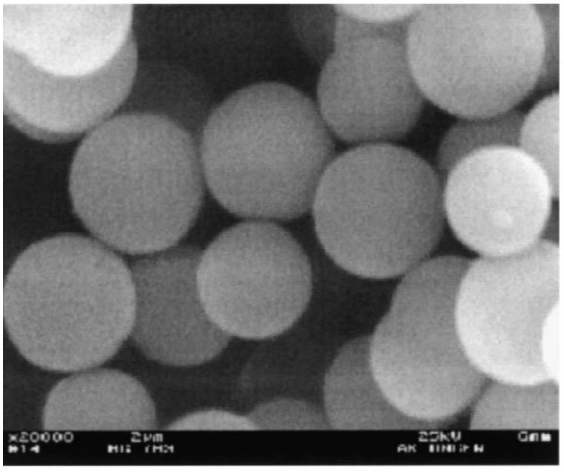
Non-porous reversed-phase silica columns (1.5–2 μm) with 33×4.6 (4.0) mm inner diameter are commercially available. Due to the low surface area of the column and the low column dead volume of about 200 μl, a number of precautions have to be



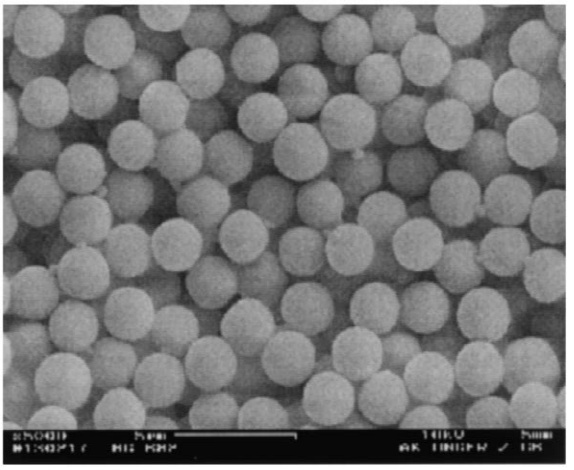
particle size :
500 nm (10000 x)



particle size :
1 μm (10000 x)



particle size :
1.7 μm (20000 x)



particle size :
2.3 μm (5000 x)

Fig. 2. Scanning electron micrographs of silica beads of various sizes, made by the amine templated synthesis route.

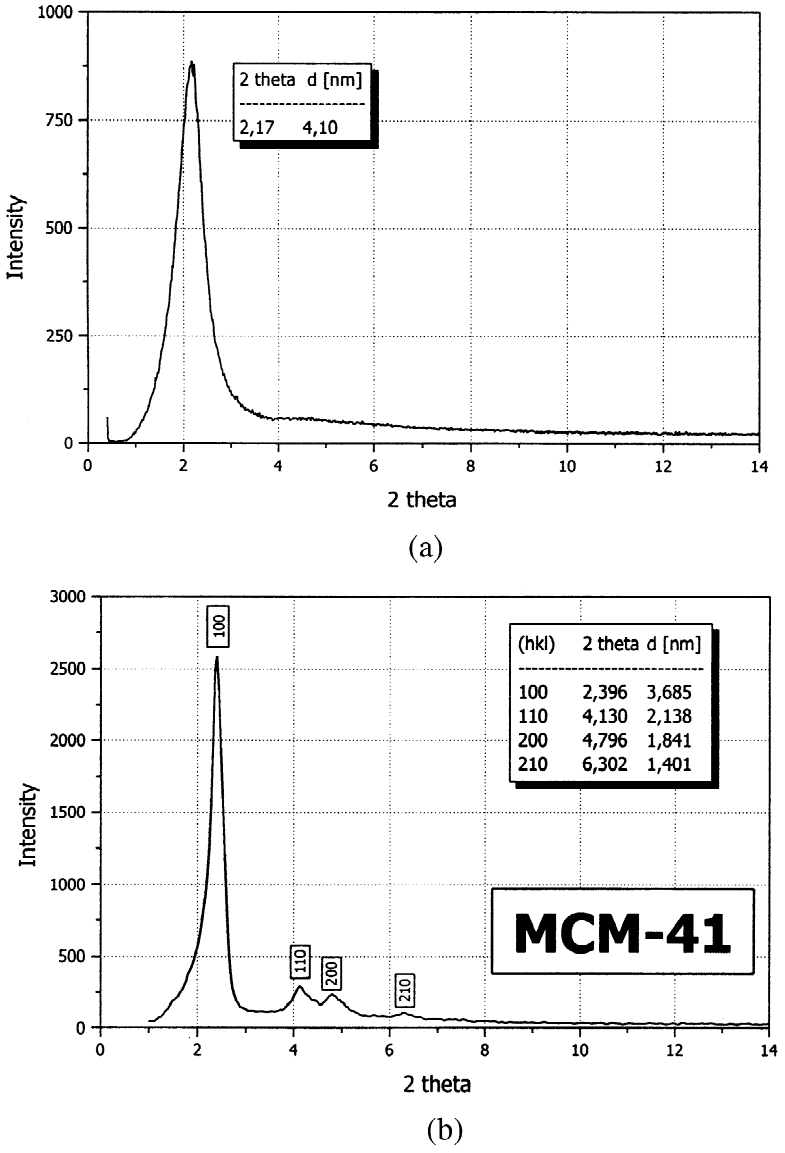


Fig. 3. X-ray diffractograms of a spherical mesoporous silica prepared according to the procedure described (a) and MCM-41 (b).

taken into account: as the specific surface area is about 100 times lower than a traditional reversed-phase silica the column loadability is also reduced by the same factor. When transferring retention protocols from porous to non-porous reversed-phase columns the water content of the eluent has to be increased with a simultaneous decrease in the content of organic solvent.

The extra column volume contributions must be minimized such as injection volume, capillary connections and volume and geometry of the detector cell to achieve the expected column performance and sensitivity. The data sampling rate of the detector should be 50 Hz to monitor fast separations with sufficient accuracy. A survey of the chromatographic application and the handling of Micra ODS columns is reported by Lamotte [31].

The most serious drawback of the 1.5 μm non-

porous reversed-phase silica column is the high column pressure drop. Even for short columns it amounts to about 150–200 bar with a flow-rate of 1–2 ml/min. It can be reduced by enhancing the column temperature to about 40°C. Table 1 displays a survey of three types of columns namely A, B and C and their chromatographic properties. Type A belongs to type of columns that are applied for routine HPLC analysis, type B is employed for fast separations and type C corresponds to columns packed with 1.5-μm non-porous silicas, as discussed above.

To overcome the pressure limitations of reversed-phase silica columns of $d_p < 3$ μm, columns with a continuous bed were introduced (SilicaRod columns, Merck, Darmstadt, Germany). These columns are composed of a continuous system of flow-through pores of about 1–2 μm and mesopores of about 10 nm [33]. The columns can be run at high flow-rates of up to 7 ml/min without any significant loss in column performance. The pressure drop is about 5 times lower than that of 5-μm reversed-phase columns at comparable conditions [34,35]. Packed capillaries with 300 μm I.D. have found applications in reversed-phase gradient elution of peptides and polypeptides but not in routine HPLC analysis.

In conclusion the most striking advantage of HPLC as a separation technique is its selectivity potential through the possible combination of stationary phase and mobile phase.

For fast miniaturized separations the major limitations are:

(i) the high pressure drop (for microparticulate columns) to generate and to maintain a hydraulic flow in parallel miniaturized devices; and
(ii) the column performance which is controlled by the eddy-diffusion (term A) and the mass transfer (term C) in the Van Deemter equation. The plate height can never be smaller than $H \sim 2d_p$, due to the A term in the case of packed microparticulate columns as well as for columns with a continuous bed.

Table 1
Characteristics of HPLC columns [32] (calculated values, the eluent is assumed to be water; the column performance of columns A–C is equal and assumed to be 10 000)

	A	B	C
L (mm)	125	60	30
I.D. (mm)	4	4	4
d_p (μm)	6	3	1.5
V_m (ml)	1.1	0.53	0.18
U_{opt} (mm/s)	2.5	5	10
f_{opt} (ml/min)	1.3	2.6	5.2
Δp (bar)	100	300	>400
Analysis time (s):			
For $k=1$	100	28	6
For $k=10$	550	150	30

3.2. Capillary electrophoresis

Capillary electrophoresis (CE) offers an outstanding column performance (see Table 2). However, the selectivity is restricted to ionic analytes. Micellar electrokinetic chromatography (MEKC) opens up great potential for non-charged analytes, but it does have a number of drawbacks. With respect to fast miniaturized separation systems CE has enormous potential: CE can be performed in small channels in microfluidic systems in a highly parallel fashion.

3.3. Capillary electrochromatography

Capillary electrochromatography (CEC) is a hybrid technique of Micro-HPLC and CE [36]. Modified CE equipment is employed with fused-silica capillaries of 400–500 mm length and 50–100 μm I.D. These capillaries are packed with 3-μm reversed-phase silicas which are usually employed in HPLC, for example, Hypersil ODS. An electrical potential of up to −30 kV is applied. Samples are injected either electrokinetically or by pressure. Detection is performed by UV. Due to the surface charge of the capillary wall and the negative charge of the surface of the silica particles, characterized by the zeta potential, an electroosmotic flow (EOF) is generated which moves the eluent towards the

Table 2
Comparison of column efficiency in HPLC, CE and CEC[a]

HPLC	$H = \underline{A\,u^{1/3}} + B/u + \underline{C\,u}$
CE	$H = \underline{B/u}$
CEC	$H = A\,u^{1/3} + \underline{B/u} + C\,u$

[a] The determining terms are underlined, where A (Eddy diffusion term) $= 2\lambda d_p$; B (longitudinal diffusion term) $= 2\gamma D_m$; C (mass transfer term) $= (k/1+k)^2 d_p^2/D_{st}$.

cathode. An isostatic pressure is applied to both sides of the capillaries to avoid bubble formation. Capillaries packed with 3 μm reversed-phase silicas generate about five times higher plate numbers than in micro-HPLC (pressure driven) under identical conditions using the same analytes. However, an increase in the column plate number by a factor of 5 is not a dramatic improvement.

CEC, however, offers the advantage of using smaller particles with an average particle diameter significantly lower than 3 μm and hence a much higher efficiency. The reason for this is that no pressure drop is generated in this electroosmotically driven system. Capillaries with 1–2 μm reversed-phase silicas were already used in some studies. Upon further reduction of the average particle diameter two questions immediately arise: (1) Is there a limit in minimum particle size with respect to maintenance of the EOF? (2) Does one get the predicted column efficiency from these columns ($H \sim 0.5d_p$)?

We found that capillaries packed with 0.2-, 0.5-, 1- and 3 μm reversed-phase silicas generate identical EOF versus field strength dependencies [37]. The experimental findings were confirmed by model calculations [38]. We also found that CEC columns packed with 0.5 μm reversed-phase silicas did not provide the expected column performance (plate height $H \sim 0.5d_p$). The plate number achieved was similar to that of 2 μm CEC capillaries at otherwise constant conditions. We assume that the EOF that was 3 mm/s was too low to operate this column under optimum conditions (about 6 mm/s).

With the current commercially available CEC equipment three major limitations exist for the operation of short capillary columns with submicron size reversed-phase silicas:

(i) the maximum potential is about −30 kV and the maximum field strength about 800 V/cm at a column length of 400 mm;

(ii) the minimum column length is about 400 mm (packed part 80 mm, unpacked part 320 mm);

(iii) the column is not thermostatically controlled.

Fig. 4 shows as an example the separation of digoxin and its metabolites in less than 2 min [39] on a CEC column packed with 0.5 μm *n*-octyl-bonded silica. In general, CEC has the potential to be downsized to microfluidic fast and high throughput separation systems. The major obstacles in this approach are:

1. the ruggedness and longevity of CEC columns do not yet meet the current requirements;
2. the parameters controlling the EOF are not yet fully understood (likewise for the conditions to achieve and to maintain a reproducible EOF);
3. there are no fundamental studies elucidating the retention and selectivity of charged and polar analytes, respectively, in CEC.

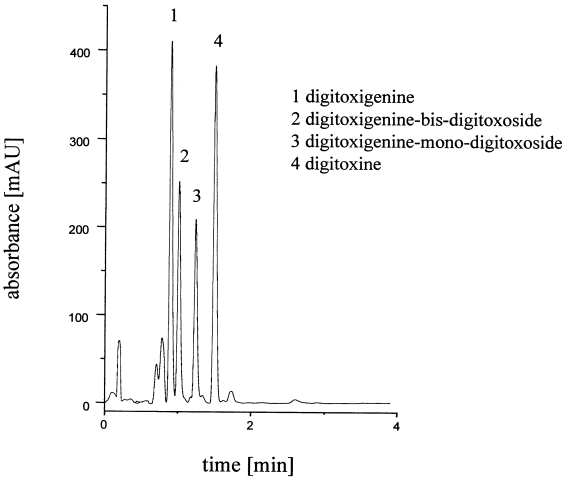


Fig. 4. Separation of four cardioactive substances. Capillary, 8.5 (38) cm×10 μm, packed with 0.5 μm C_8; mobile phase, acetonitrile–12 m*M* Tris–HCl, pH 6 (60:40), 30°C, −790 V/cm; detection, 254 nm UV.

4. Conclusion

Among the chromatographic and electrokinetic separation methods CE and CEC bear the largest potential to become the powerhouses in fast miniaturized separation systems. CEC as compared to CE has the advantage of being an orthogonal method to CE and micro-HPLC. CEC is applicable to both ionic and nonionic analytes. It should be emphasized that the success of miniaturized separation systems is by and large determined by the design and development of highly efficient sample clean-up systems.

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