Evaluation of Polymeric Methacrylate-based Monoliths in Capillary Electrochromatography for their Potential to Separate Pharmaceutical Compounds

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Abstract

Polymeric methacrylate-based monoliths are evaluated in capillary electrochromatography (CEC) and pressurized capillary electrochromatography (p-CEC) for their potential in pharmaceutical analysis. Using a given polymerization mixture as a basis for the monolith synthesis, different mobile phase pH at constant organic modifier concentrations are tested in both CEC and p-CEC. The test set consists of basic, acidic, amphoteric, and neutral compounds, which are mainly pharmaceuticals. Because of the mainly hydrophobic character of the stationary phase, the interactions are largest when the compounds appear in an uncharged state, but some ion-exchange phenomena with negatively charged compounds can also be observed. In CEC, acidic substances are most retained at low pH. For amphoteric and neutral compounds, no preference regarding analyzing pH can be derived from these experiments. For basics, a high pH is chosen, but a reduced solvent strength is needed to enhance the retention of these compounds. The retention mechanism in p-CEC can also be assigned to both hydrophobic and ionic interactions. For acidic, amphoteric, and neutral compounds, acceptable retention is seen. For the basic compounds, the retention with a mobile phase containing 50% organic modifier is low, as in CEC. However, when the organic modifier content in the mobile phase is decreased, retention increases and the selectivity of the stationary phase is more pronounced. This mode of operation presents a possibility for separating some test mixtures, thus some potential for pharmaceutical analysis is seen. More efforts are needed to obtain higher efficiencies and better peak shapes, which might be solved by a further optimization of both the stationary phase synthesis and the mobile phase composition.

Introduction

Capillary electrochromatography (CEC) is a separation technique that uses capillary chromatographic columns through which the mobile phase is driven under the influence of an electrical field. The driving force of the mobile phase (the electroosmotic flow) has a plug-like form and gives rise to sharp peaks, leading to more efficient separations as compared with liquid chromatography (1,2). Because of a combined separation principle [i.e., electrophoretic migration and chromatographic retention (3)], new selectivities can possibly be obtained, compared with the commonly used separation techniques, high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE).

In theory, the properties of CEC sound very promising for separation science. However, in practice, some experimental problems occur that prevent CEC from being suitable for application in an industrial environment, which is the ultimate goal for every separation technique. A main drawback of CEC is the lack of commercially available columns and stationary phases (3). As a consequence, the analyst has to prepare them in-house, which can be detrimental for the between-column repeatability (4,5). Most CEC analyses up till now have been performed using particle-based stationary phases inside the capillary (6–10). Regarding the type of stationary phases, one can choose to perform analyses using functionalized particles of phases designed for HPLC. However, column lifetime will be reduced because these phases are not developed for the application of an electrical field. Also, column performance can significantly decrease as a function of time. Another disadvantage associated with particle-based stationary phases is the need for frits to fix the stationary phase inside the capillary. Frits are responsible for column fragility, and can also cause bubble formation during analysis, leading to current disruption, current breakdown, and noisy baselines (11). They can also loosen under the influence of an electrical field, leading to column failure.

As previously described, it is seen that the use of particle-based stationary phases presents a possibility for CEC analysis, but is characterized by several drawbacks. One of these drawbacks (i.e., the presence of frits) can be resolved when continuous media bonded onto the capillary wall, the so-called monolithic stationary phases, are used.

Monoliths can be divided into two major categories, silica-based (12,13) and polymer-based (14,15). The former are created

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after a sol gel reaction of alkoxysilane precursors in a capillary. The latter are created after a polymerization reaction between monomers and cross-linkers inside the capillary. Because of pH restrictions associated with silica-based monoliths and the more complex preparation of the matrix, it was chosen in this study to evaluate polymeric monoliths as an alternative for particle-based stationary phases. First, methacrylate-based monoliths (16–19) were tested. Literature mostly describes the performance of this type of monoliths using neutral model compounds that are analyzed relatively easy (16,20). However, analyses of other types of compounds, such as amino acids, small peptides, proteins, and aromatic carboxylic acids (21) are also reported. This study aimed at evaluating these monoliths for pharmaceutical analysis.

Using a test set that contains acidic, basic, neutral, and amphoteric compounds, of which most are drug molecules, different experimental conditions were tested on columns obtained from a given polymerization mixture. Analysis conditions were searched for where acceptable retention was seen and where different selectivities of the stationary phase towards the compounds were displayed.

The columns were tested in both CEC and pressurized CEC (p-CEC) mode. The latter can be seen as CEC where the mobile phase is delivered by pumps at the inlet vial (22–24). The resulting flow profile is the sum of a parabolic (pressure-driven) and plug-like (electrical-driven) flow. The obtained efficiencies are somewhat lower than in regular CEC. However, some practical problems such as column drying, bubble formation inside the capillary, and injection issues (the system uses a loop injection) can be resolved using the p-CEC mode. It was therefore evaluated whether p-CEC can provide additional benefits over regular CEC.

Table I. Test Set Compounds and their Numbers Used in the Figures*

No	Compound	No	Compound
1	Acenocoumarol	28	o-terphenyl
2	Acetyl salicylic acid	29	Phentolamine
3	Captopril	30	Methyldopa
4	Chlorthalidone	31	Oxazepam
5	2,5-di-OH-benzoic acid	32	Temazepam
6	Di-OH-naphtalene	33	Tetracycline
7	Fenoprofen	34	Alprenolol
8	Flurbiprofen	35	Betaxolol
9	Hexobarbital	36	Ambucetamide
10	Ibuprofen	37	Atropine
11	Methylphenobarbital	38	Bupranolol
12	Naproxen	39	Cinnarizine
13	Paracetamol	40	Clonidine
14	Sulindac	41	Coffein
15	Suprofen	42	Diazepam
16	Warfarin	43	Dilthiazem
17	Biphenyl	44	Ephedrin
18	Chloramphenicol	45	Fluoxetin
19	Felodipine	46	Lidocaine
20	Phenazon	47	Metoprolol
21	Naphtalene	48	Nicotinamide
22	Nimodipine	49	Pindolol
23	Nitrendipine	50	Prazosin
24	Phenantrene	51	Procaine
25	Piracetam	52	Sulpiride
26	Praziquantel	53	Thiamine
27	Pyrene	54	Verapamil

* 1–16: Acidic; 17–28: Neutral; 29–33: Amphoteric; 34–54: Basic.

Materials and Methods

Capillary electrochromatography

CEC experiments were performed using a Beckman P/ACE MDQ CE system (Fullerton, CA), controlled by the Beckman 32 Karat software version 4.01 (1999–2000 Beckman Coulter) and equipped with a diode array detector, which was set at 214 or 254 nm. During analysis, the temperature was kept constant at 25°C by means of liquid cooling. All analyses were performed at –15 or –10 kV. Pressurized injections were performed using 3.0 psi, and electrokinetic ones at –5 kV. The injection times varied from 5 to 30 s. During analysis, a pressure of 80 psi was set on both vials. In these experiments, columns with a total length of 31.2 cm and a 21 cm monolith section were used. Columns were conditioned before first use by applying –5, –10, –15, –20, and –25 kV for 10 min each. When mobile phases were changed, the columns were rinsed for one hour using a flow-splitted HPLC pump (Merck-Hitachi, Tokyo, Japan)

Pressurized capillary electrochromatography

p-CEC experiments were conducted using a Trisep 2100 GV p-CEC system (Unimicro Technologies, Pleasanton, CA). Data acquisition was performed by means of Unimicro workstation software version 2.12 (Unimicro Technologies). A flow of 0.1 mL/min was applied during analysis, which generated a backpressure of around 70 bars, and which was controlled by a backpressure regulator. The detector was set at 214 nm for the compounds of the test set, and 254 nm for the determination of the dead time, because acetone hardly absorbs at 214 nm. The applied voltage was set either at –14.4 or at –5 kV. A loop of 2 µL was used for injection. However, only a fraction (nanoliters) was injected onto the column due to the backpressure regulator, which can split the flow of the mobile phase before entrance into the column. Experiments to determine the exact injection volume were not executed. The columns used had a total length of 45 cm, of which 20 cm was filled with monolith. Columns were conditioned at first use by applying –5, –7, and –10 kV for 10 min each. When mobile phases were changed, the columns were equilibrated using a flow of 0.1 mL/min.

Samples and reagents

To prepare the samples and mobile phases, Milli-Q water produced in-house by a Milli-Q Water Purification System (Millipore, Milford) and HPLC grade acetonitrile (ACN) from VWR (Leuven, Belgium) were used. Samples were dissolved at a concentration of 0.5 mg/mL in a mixture of ACN–Milli-Q (50:50, v/v). When the compounds were insufficiently soluble, a higher ACN fraction was used. The sample solutions were ultrasonicated

for 20 min and kept in the refrigerator. The test set consisted of 16 acidic, 12 neutral, 5 amphoteric, and 21 basic substances. Their names and numbers used in the figures are displayed in Table I. As EOF marker, a solution of 20% (v/v) acetone (Merck) in (50:50, v/v) Milli-Q–ACN was used.

For the preparation of a 50mM phosphate buffer pH 7, sodium dihydrogen phosphate ($NaH_2PO_4.12H_2O$) and disodium hydrogen phosphate ($Na_2HPO_4.H_2O$) of Merck (Darmstadt, Germany) were used. A 200mM ammonium formate buffer pH 3 was prepared using formic acid 98–100%, pro analyse (Merck). A 50mM phosphate buffer pH 11.5 was prepared using trisodiumphosphate ($Na_3PO_4.12H_2O$) (Merck). Phosphate buffers 50mM, pH 2 and 3 were prepared starting from ortho-phosphoric acid, 85% (Fluka, Buchs, Switserland). A 50mM carbonate buffer pH 11 was prepared from ammonium hydrogen carbonate (Fluka). The 50mM phosphate buffers pH 3 and pH 7 were brought to the required pH using 1M NaOH (Carlo Erba, Val de Reuil, France), the 200mM ammonium formate buffer using a 25% ammonia solution (Merck) and the 50mM phosphate buffer pH 11.5 and pH 2 using 1M HCl (Merck). All electrolyte solutions were diluted to the required concentration before use. The reported concentrations in this study are these in the total mobile phase (i.e., the mixture of aqueous solution and ACN). For each mobile phase tested, a fraction of 50% (v/v) ACN was used.

Butyl methacrylate (99%, BMA), ethylene dimethacrylate (98%, EDMA), 1,4-butanediol, 1-propanol, and [2-(methacryloyl)ethyl]trimethylammoniumchloride (75%, META) were purchased from Sigma (St. Louis, MO). 2,2'-Azobisisobutyronitrile (AIBN) was purchased at Fluka.

Column preparation

Prior to the polymerization, the capillary wall of a 100 µm i.d. capillary (Composite Metal Services, Ilkley, UK) was surface-modified with 3-(trimethoxysilyl)propyl methacrylate (Aldrich, Milwaukee, WI). Then the capillary was filled by means of a syringe with a polymerization mixture composed of BMA and EDMA in a 60:40 w/w ratio. The polymerization mixture further contained 22% (w/w) 1,4-butanediol, 52% (w/w) 1-propanol, and 6% (w/w) of a 10-fold dilution of META 75% in water. The initiator of the reaction was AIBN. Before the capillary was filled, the mixture was ultrasonicated and purged with nitrogen, each for 10 min. The polymerization reaction was conducted at 70°C for 20–24 h. After polymerization, unreacted monomers and pore-forming solvents were removed by rinsing the columns with pure methanol (HPLC grade, Fisher Scientific, Leicestershire, UK). A detection window was created next to the monolithic bed by means of a capillary burner (Electro-Kinetic Technologies, Broxburn, UK).

Calculations

The retention behavior of substances on the column was evaluated by means of a peak locator, which is calculated according the chromatographic formalism suggested in the literature (25):

$$k''_{cc} = \frac{t_m - t_0}{t_0} \qquad \text{Eq. 1}$$

where t_m indicates the elution time of the analyte and t_0 the migration time of a neutral and inert marker in CEC. As it can be seen, the calculation of the peak locator is identical to the one of the retention factor (k) in chromatography. Therefore, the terminology "retention factor" will be further used in this study. The only difference between k and k''_{cc} is that one obtains negative k-values for compounds that are eluting faster than the marker.

Elution times, number of theoretical plates, and peak asymmetry were determined by the software of the instruments.

Results and Discussion

The surface of the used polymeric stationary phase is hydrophobic. Therefore, most interactions of the substances with the monolithic phase are expected when they are in their most hydrophobic (i.e., uncharged state). Due to the presence of META in the polymerization mixture, positively charged sites are present inside the capillary, enabling electrochromatographic experiments. Thus, besides the hydrophobic partition, some ion-exchange properties of the positively charged stationary phase can also be expected with negatively charged species, thus with acidic and amphoteric substances at higher pH. However, it is not our intention to make primary use of this interaction as a retention mechanism because it does not occur for all substances, and such a situation is mostly avoided when searching for general analysis conditions.

All mobile phases initially contained 50% (v/v) ACN. The ratio of organic modifier was decreased to 40 and 30% when its effect on the retention was investigated. As the experiments performed were considered preliminary, no further optimization of the parameters was performed yet.

The analyzing voltage in CEC was chosen in such a way that a dead time around 5 minutes was obtained, which was mostly at –10 kV. For p-CEC the analyzing voltage was set on –5 kV, because here the mobile phase flow was also supported by pressure.

The test set used contained 54 compounds. The majority of these compounds are drug molecules, because this study's main aim was to evaluate the monoliths for their application in pharmaceutical analysis. Additionally, some polyaromatic hydrocarbons were also included, as they are frequently used as model compounds in separation science.

CEC experiments

A high number of injections had to be performed due to the large test set used, and it was observed that columns were sometimes broken throughout a set of experiments. Breakage of columns results partly from the fact that the column ends are in contact with vials, which are moved when performing injections and changing buffer vials. Therefore, a new column, which was made from the same polymerization mixture, was used with each mobile phase investigated. Even though the columns were synthesized from the same mixture, slight changes in the morphology of the bed can occur, which can lead to some between-column variability in the results.

The first CEC experiments were conducted using a 5mM phos-

phate buffer pH 7 as electrolyte and –15 kV as analyzing voltage. First, pressurized injections were used. When no peak was observed using pressure, an electrokinetic injection was tried. For 11 compounds, no peak was seen using either of the injection types. For the other compounds, a signal was observed for at least one injection type, although interpretation of the results was not always straightforward. Signals were sometimes very low, making it difficult to decide whether the observed peak was the injected compound. Figure 1 shows the retention factors that were obtained using these analyzing conditions, as a function of molecular weight (A) and the acidic-basic properties of the compounds (B). The retention factor can be considered as independent from the molecular weight (i.e., no trend between the size of the molecule and its retention was seen). Apparently, retention is mainly dominated by the presence of polar groups. This lack of tendency was observed for all mobile phases and experimental conditions tested, so it will not be discussed further. Possibly, this effect becomes more significant when other compositions of the polymerization mixture are tested, resulting in stationary phases with smaller pore sizes.

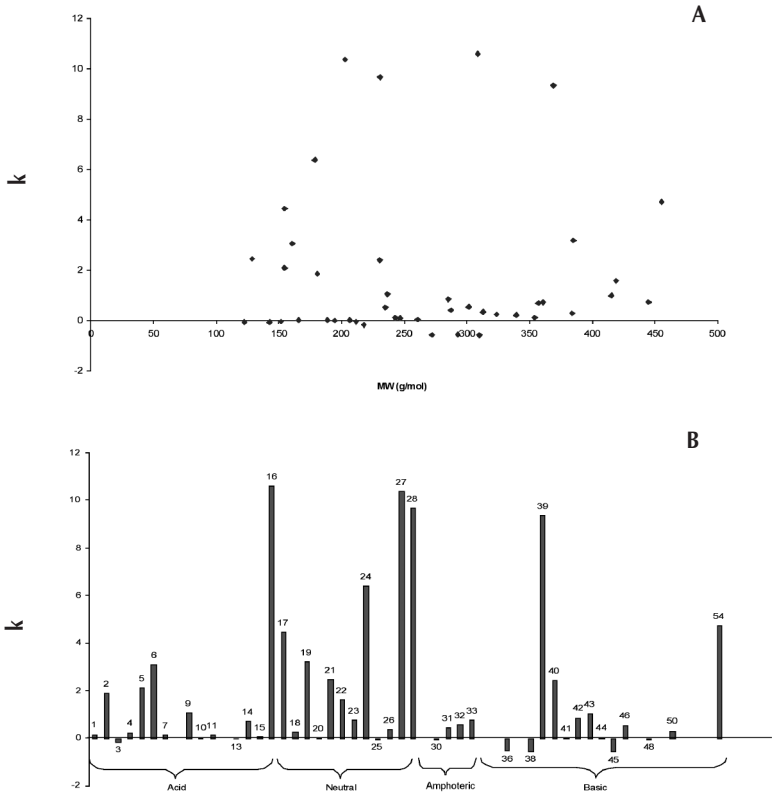

Figure 1. Retention factors of 54 compounds in CEC as a function of molecular weight (A) and acidic-basic properties (B). Mobile phase: 5mM phosphate buffer pH 7/ACN (50/50), –5 kV; injections: 3 psi, 5–30 s or –5 kV, 5–30 s; detection at 214 nm. When a compound number was not indicated, it was not observed/detected within 60 min.

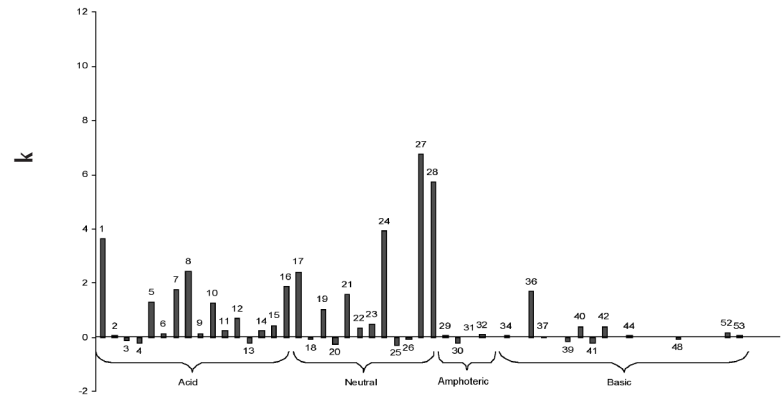

Figure 2. Retention factors of 54 compounds in CEC. Mobile phase: 20mM ammonium formate buffer pH 3/ACN (50:50), –10 kV; injections: –5 kV, 30 sec; detection at 214 nm. When a compound number is not indicated, it was not observed/detected within 60 min.

It was also decided to use electrokinetic injections (–5 kV, 30 s) in further experiments, as they were sometimes able to inject a compound when the pressurized injection was not.

Regarding the retention as a function of the acidic, basic, amphoteric, or neutral character of the compound, it was seen that the neutral compounds were best retained, followed by the acidic species. For the latter, ionic interaction with the positively charged stationary phase probably plays a role in the retention mechanism. For amphoteric and some basic compounds, moderate retention is seen. For most basic compounds, no injection or a very fast elution was seen. The latter is probably caused by repulsion by the stationary phase. This can also explain the negative retention factors seen for some compounds (i.e., when the compound is repulsed by the stationary phase, it probably moves faster than the EOF).

In the next step, a low pH 20mM ammonium formate electrolyte (pH 3) was used at –10 kV, with the intention of keeping the acidic compounds uncharged. Figure 2 shows the retention factors obtained as a function of the classes. All acidic compounds eluted and more were retained than at neutral pH. This was expected, as the hydrophobic interaction with the stationary phase should increase. It also can be seen that the acidic compounds which were retained at pH 7 now were less retained at a low pH. Probably, their ionic interaction with the stationary phase when they are negatively charged is higher than their hydrophobic interaction when they are uncharged. Another factor that can cause this reduced retention is that the analyses at high pH

were performed on another column, thus between-column variability issues can play a role. The results observed above indicate a changed retention mechanism at both pH. For some neutral compounds such as pyrene, o-terphenyl, and phenantrene, a decrease in the retention factor was seen as compared with the results obtained at neutral pH, which can be assigned again to between-column variability. For basic and amphoteric compounds, the number of compounds without retention or with fast elution is even more pronounced at low pH, because they are fully positively charged.

A problem with this electrolyte was the instability of the current. This had an influence on the obtained elution times. A possible explanation is that the concentration of ammonium formate was not high enough to provide a constant current. Therefore, it was increased to a total concentration of 50mM, and compounds 1, 2, 4, 9, 15, 21, 22, 27, 30, 32, 37, 38, 43, 44, 46, 48, 52, and 54 were re-analyzed on a new column. Verapamil (54), dilthiazem (43), lidocaine (46), ephedrin (44), sulpiride (52), and bupranolol (38) were not detected using the 50mM electrolyte. The results with both concentrations were relatively comparable for the majority of the compounds, except for naphthalene, pyrene, and acenocoumarol. For naphthalene and pyrene, a difference of 1–1.5 in retention factor was observed between both concentrations of the electrolyte. For acenocoumarol, a difference (0.5–1) in retention factor was also seen with both concentrations of electrolytes. It was also observed that ephedrine and sulpiride only eluted when a 20mM electrolyte was used. The differences in the retention behavior of neutral compounds can be explained by the fact that a new column with the 50mM electrolyte was used, which can display slight changes in morphology. Because the current was more constant at 50mM, this concentration was preferred.

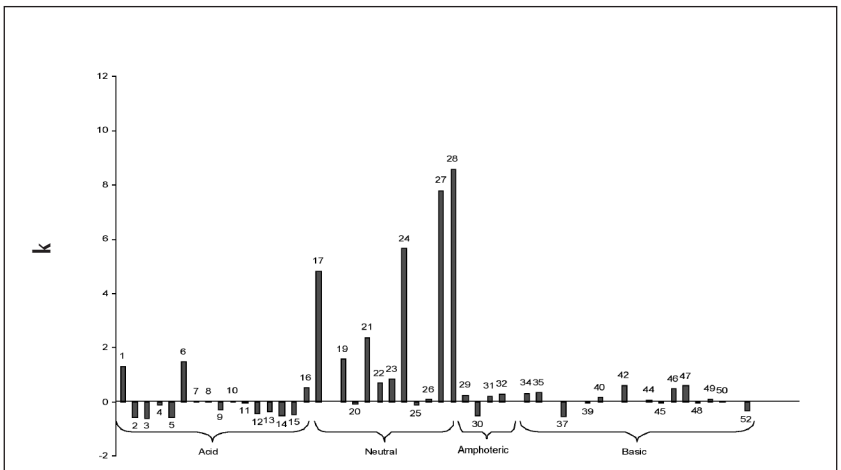

Figure 3. Retention factors of 54 compounds in CEC. Mobile phase: 5mM phosphate buffer pH 11.5/ACN (50:50); other conditions as in Figure 2. When a compound number was not indicated, it is not observed/detected within 60 min.

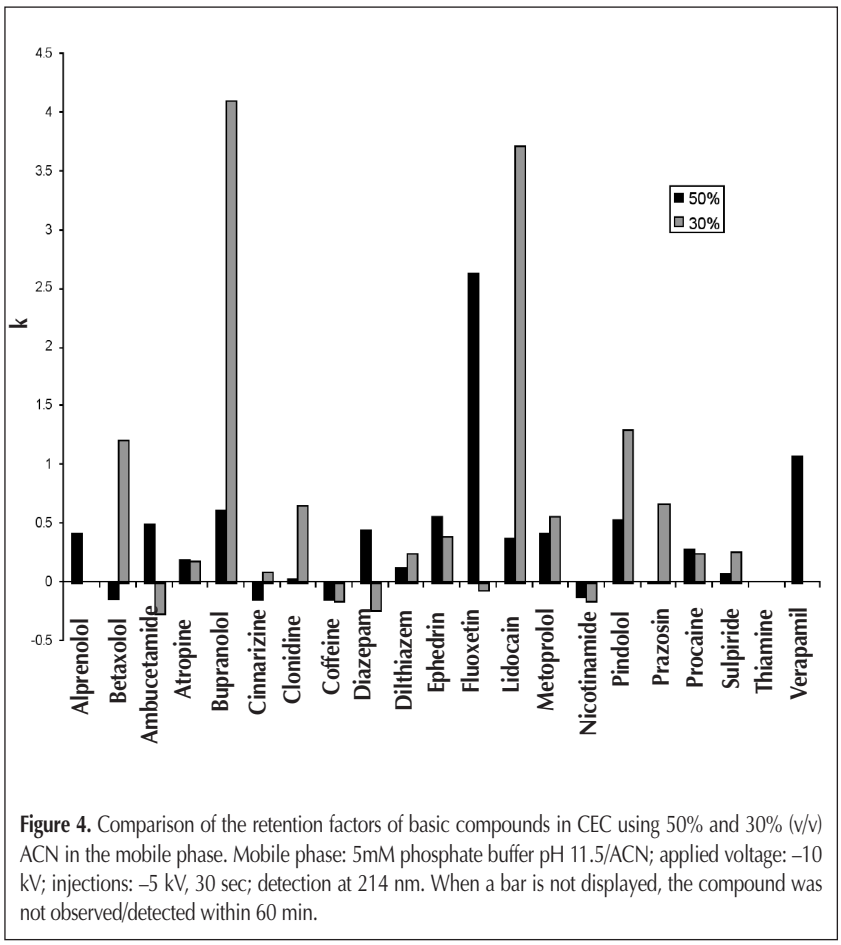

Figure 4. Comparison of the retention factors of basic compounds in CEC using 50% and 30% (v/v) ACN in the mobile phase. Mobile phase: 5mM phosphate buffer pH 11.5/ACN; applied voltage: –10 kV; injections: –5 kV, 30 sec; detection at 214 nm. When a bar is not displayed, the compound was not observed/detected within 60 min.

The between-column variability was also tested with the 50mM electrolyte. From the results displayed in Table II, it was seen that (except for acenocoumarol) the retention factors were relatively comparable, indicating relatively good repeatability between different columns for this parameter. For the elution times, however, large variations were observed. For example, the elution time for acenocoumarol was 14.9 min on the first column, but 23.5 min on the second one. Also, the dead time of the second column was about 1 min higher than the one of the first columns. Possibly, the monolithic structure is somewhat different in both columns (i.e., the pore and globule size of the second column appears to be smaller, resulting in a lower flow inside the column and systematic higher elution times). Overall, retention factors give more repeatable values compared with elution times.

A phosphate buffer at pH 11.5, to keep the basic compounds uncharged and to study the effect of a high pH on the retention of all compounds, was used on the complete set (see Figure 3). For acids, some retention was expected using this mobile phase because of the ionic interaction with the positively charged stationary phase, cor-

responding with what was seen with the phosphate buffer at pH 7. However, less retention was seen compared with pH 7 and pH 3. Possibly, the electrophoretic mobility of the negatively charged compounds towards the anodic electrode dominates the ionic interactions. For neutrals, a similar retention was observed as with the previous mobile phases. For amphoterics, somewhat more retention was seen than at low pH, but less than at pH 7. However, retention was limited. For the basic compounds, the results were somewhat disappointing. No spectacular retention changes were observed and the retention factors for several compounds were even higher at pH 7 than at pH 11.5. This is unexpected, as the interaction with this stationary phase is supposed to be highest for the uncharged basic compounds.

Table II. Between-Column Variability in Elution Times and –Factor on Two Columns*

Substance	Column 1		Column 2	
	t_m	k	t_m	k
Acenocoumarol (1)	14,90	2,11	23,54	3,08
Acetylsalicylic acid (2)	5,39	0,12	7,20	0,25
Chlorthalidone (4)	4,92	0,03	6,02	0,04
Hexobarbital (9)	5,67	0,18	7,00	0,21
Suprofen (15)	7,16	0,49	9,38	0,62
Naphtalene (21)	21,08	3,40	23,20	3,02
Nimodipine (22)	8,92	0,86	10,68	0,85
Pyrene (27)	42,20	7,81	53,08	8,20
Methyldopa (30)	4,55	–0,05	6,06	0,05
Temazepam (32)	6,12	0,28	7,86	0,36
Atropine (37)	4,92	0,03	6,50	0,13
Nicotinamide (48)	4,97	0,04	7,51	0,30

* Verapamil, dilthiazem, lidocaine, ephedrine, sulpiride and bupranolol were not observed within 60 min. Analysis conditions: 50mM ammonium formate buffer pH 3–ACN (50:50), –5kV, 30 s injection, applied voltage: –10 kV, detection at 214 nm.

Other electrolytes, such as 5mM phosphate buffers at pH 2 and 3, and 5 and 10mM carbonate buffers, both at pH 11, were tested with an analyzing voltage of –10 kV, but no constant current could be obtained during analysis or no peaks of the compounds were observed. Therefore, these results will not be discussed further.

Generally, peak efficiencies were relatively poor with the tested mobile phases (i.e., the obtained theoretical plate numbers were below 5000, corresponding to efficiencies below 25000 plates/m). Compared with reported efficiencies of other studies of up to 170000 plates/m on basic drug molecules (26) and 140000 plates/m for neutral substances (27) with similar types of monoliths, this seems quite low. Regarding efficiencies, no preference to one or another electrolyte could be given. The peaks were also frequently tailing, with tailing factors up to 10. For compounds that were poorly retained, smaller tailing factors were seen. Occasional peak splitting also occurred in some analyses. However, no explanation for these phenomena can be given at this point.

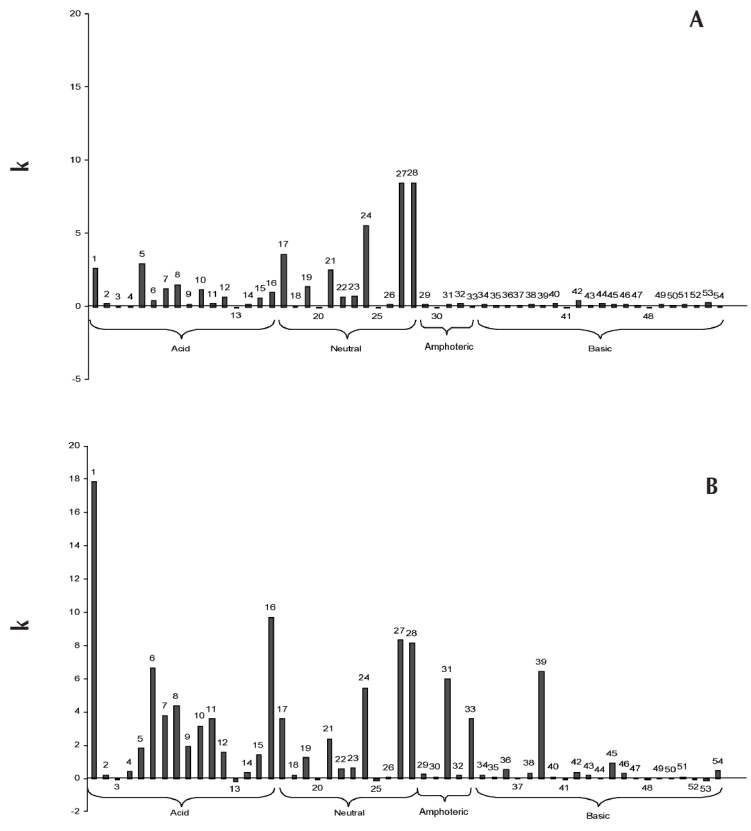

Figure 5. Retention factors of 54 compounds in p-CEC. Mobile phase: 50mM AF pH 3.00–ACN (50:50, v/v) (A), 5mM phosphate buffer pH 11.5–ACN (50:50) (B); Other conditions: Applied voltage: –5 kV; Flow: 0.100 mL/min; Detection at 214 nm.

The CEC experiments with this type of monolithic phases provide some possibilities. It was seen that for acidic compounds, both hydrophobic and ionic interactions can occur, the latter when the substances are in a charged state. However, the best retention for these compounds was observed when a low pH electrolyte was used, therefore, this was preferred. At high pH, the electrophoretic mobility probably dominates the ionic or hydrophobic interactions and fast elution is observed. For neutral compounds, retention was observed at all pH values, which is quite logical as they remain uncharged at any pH. Therefore, no preference exists regarding pH analysis. For amphoteric compounds, the most (but limited) retention was seen at pH 7, followed by pH 11.5. At low pH, hardly any retention was observed, which can be explained by the repulsion of the stationary phase for the positive charges. Therefore, low pH must be avoided with these compounds. Finally, for most basic compounds, hardly any retention was seen at any pH, most retention of basics was observed at neutral pH. Possibly, the elution strength of the tested mobile phases was too high to allow interaction of these compounds with the stationary phase. Therefore, additional experiments were conducted using a

high pH mobile phase that contained 30% (v/v) ACN. The results are displayed in Figure 4. No general trend could be derived from the decrease of elution strength of the mobile phase. Thiamine did not elute in either mobile phase. For 13 compounds, a similar or an increase in retention factor was seen, which is the expected outcome. For 7 compounds, the retention factor decreased or no signal was observed within 60 min. Therefore, we can conclude that for the majority of the compounds, an increase in retention factor was seen. For basic compounds, a high pH seems recommended when one takes into account that at lower pH values the positively charged bases will be repulsed by the stationary phase. A decrease of the organic modifier content might provide a solution when the compounds are not retained.

p-CEC experiments

Pressurized CEC is a variant of CEC in the sense that a pressure-driven flow is provided at the column inlet, while an electrical field is applied over the column. Because extra flow is provided by means of the pump, the applied electrical field in the experiments was set at only –5 kV to avoid too fast of an elution. Because of the trends seen in the CEC experiments, only a low pH ammonium formate buffer and a high pH phosphate buffer were selected for the analyses. The retention factors obtained in p-CEC at low and high pH are displayed in Figures 5A and B, respectively. All compounds were injected by means of a loop injection system, which guaranteed a proper sample injection. Because the columns are fixed inside the p-CEC instrument and are not exposed to moving vials, the risk of breaking the column throughout analysis is reduced. Therefore, all experiments in p-CEC could be executed on one single column.

For acidic compounds, more retention was observed at high than at low pH, which is probably caused by the ionic interaction with META. Here, no loss of retention was seen due to an increased electrophoretic mobility towards the anode at pH 11.5 as in CEC. This can be explained by the lower analyzing voltage that was used in the experiments. In the context of defining general starting conditions to analyze pharmaceutical compounds, it is preferable that only one interaction principle is used for all compounds. For acidic compounds, one can benefit from additional ionic interaction, which can increase the retention of these compounds. However, this interaction cannot be present for basic compounds, as only repulsion can be observed when they are charged. Therefore, the hydrophobic interaction mechanism is preferred. Regarding analyzing conditions for acidic compounds, our preference goes to an acidic mobile phase to be used first, where the ionic interaction does not occur. If needed, a high pH can be tested for a specific mixture or compound when the low pH mobile phase does not provide the desired results.

For neutral compounds, similar retention was observed at both pH, which is to be expected. For two amphoteric compounds, more retention was seen at high pH, which can again be related to the ionic interaction with the stationary phase at high pH and the repulsion at low pH. These types of interactions cannot be excluded for amphoteric compounds, and interaction was preferred over repulsion, therefore pH 11.5 was preferred.

For basic compounds, hardly any retention occured at low pH. At high pH, some retention for a limited number of compounds was observed, but some optimization of the analyzing conditions was needed to further enhance their retention. To increase the retention of basic compounds, the elution strength of the mobile phase was reduced. Figure 6 shows that the retention factors indeed increased when the content of ACN in the mobile phase was decreased to 40 or 30% (v/v). A larger variation of retention factors was observed at 30% (v/v) ACN, indicating more selectivity differences of the stationary phase towards the compounds. It must also be mentioned that a decrease in organic modifier content had an enormous effect on the number of theoretical plates obtained (per column): they decreased from 500–1000 with 50% ACN to less than 100 with 40 and 30% ACN.

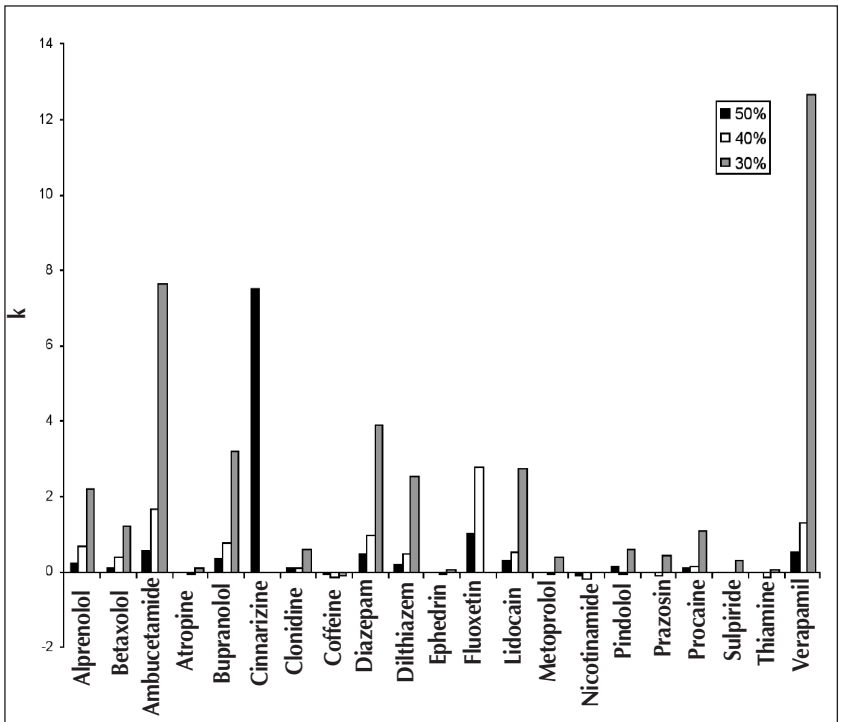

Figure 6. Effect of the ACN content on the retention factors of basic compounds in p-CEC. Mobile phase: 5mM phosphate buffer pH 11.5/ACN [%ACN (v/v) specified in figure]; applied voltage: –5 kV; flow: 0.100 mL/min; detection at 214 nm. When a bar is not displayed, the compound was not observed/detected within 60 min.

The p-CEC mode allowed the finding of more general trends regarding mobile phases to be used (i.e., low pH for acids and high pH for basic, amphoteric, and neutral species), and confirmed what was expected. Three artificial test mixtures of acidic, basic, and neutral compounds, respectively, were analyzed. The resulting electrochromatograms are given in Figure 7. As it can be seen, a relatively good separation could be obtained for the analyzed mixtures. However, it can also be observed that the peaks are broad, which was also reflected in the low theoretical plate numbers obtained, as discussed previously.

Changes in the polymerization mixture to synthesize the monoliths can possibly give rise to stationary phases with better efficiencies.

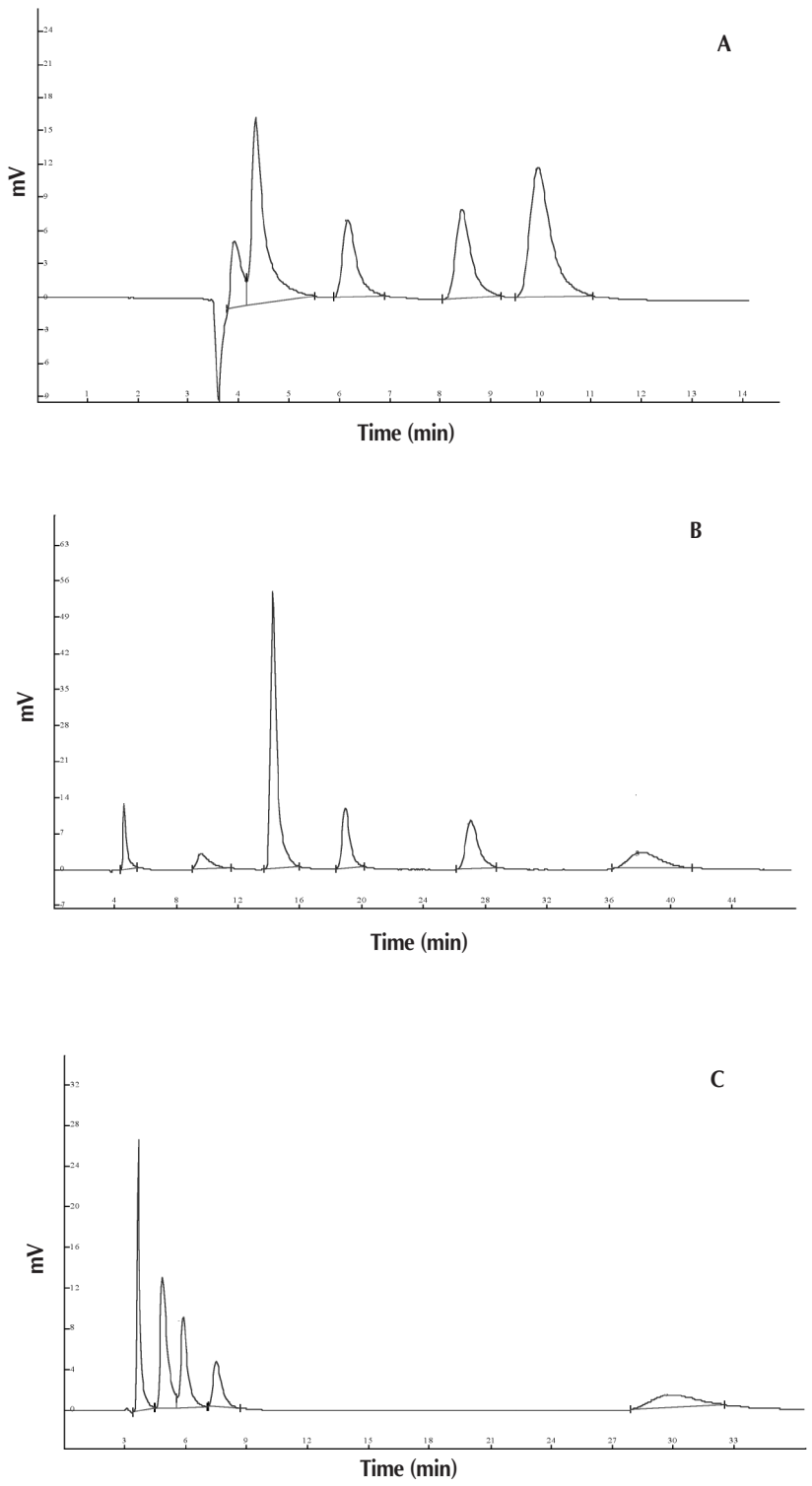

Figure 7. Separation of mixtures consisting of captopril (3.902 min), sulindac (4.325 min), suprofen (6.155 min), ibuprofen (8.418 min), and flurbiprofen (9.930 min); conditions as in Figure 5A (A); praziquantel (4.532 min), felodipine (9.492 min), naftalene (14.158 min), biphenyl (18.846 min), phenantrene (26.933 min), and *o*-terphenyl (37.971 min); conditions as in Figure 5A (B); nicotinamide (3.622 min), fentolamine (4.823 min), verapamil (5.848 min), fluoxetine (7.472 min), and cinnarizine (29.790 min); conditions as in Figure 5B (C).

When a comparison is made between the p-CEC and CEC results, we can clearly see that p-CEC presents some advantages: there were no injection problems and the theoretically expected retention mechanism was more pronounced. However, regarding theoretical plate numbers obtained, both modes provide similar results.

Conclusion

The retention of different classes of compounds on methacrylate polymeric monoliths applying regular and pressurized capillary electrochromatography was investigated using columns synthesized from a given polymerization mixture. Different types of electrolyte at different pH were tested as the mobile phase, and their effect on the retention was evaluated.

In CEC, the acidic species were most retained when a low pH mobile phase was used. Amphoteric and neutral compounds were retained at both neutral and high pH, and no preference regarding pH analysis could be expressed. For basic compounds, a high pH is recommended, although retention was low using 50% (v/v) of organic modifier in the mobile phase. For some compounds, more retention was achieved by decreasing the organic modifier content in the mobile phase. Also, experimental problems occurred in CEC because some compounds could not be injected.

In p-CEC, the loop injection system of the device allowed the injection of all compounds. In p-CEC, more general trends regarding mobile phases to be used could be derived (i.e., a low pH for acids and high pH for other species). A decrease in organic modifier content to increase the retention of the basics had the expected effect more clearly than in CEC. The compounds were better retained, however, at the cost of peak efficiencies. p-CEC also allowed the separation of some artificial test mixtures. Therefore, we can state that this mode already showed some potential for separating pharmaceutical substances.

Regarding of column performance, efforts are needed in the future to enhance the obtained efficiencies and peak shapes. This also applies to CEC analysis.

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