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Influence of the polymerization-mixture composition for monolithic methacrylate-based columns on the electrochromatographic performance of drug molecules

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Abstract

Methacrylate-based monolithic stationary phases were evaluated for the analysis of drug molecules using capillary electrochromatography (CEC) as separation technique.

The effect of the polymerization-mixture composition on the retention behavior of a small test set of mainly drug molecules was studied. Two factors were varied in a central-composite design-based approach: the ratio between the pore-forming solvents and the monomers on one hand, and the ratio within the pore-forming solvents on the other hand, resulting in nine different stationary phases. The central point of the design was chosen at 70% (m/m) pore-forming solvents (PFS) of which 30% (m/m) is 1,4-butanediol, i.e. 21% of the total polymerization mixture. Experiments were conducted using both a basic (pH 11.5) and an acidic (pH 3) mobile phase. Retention times, retention factors, peak asymmetry and number of theoretical plates are the responses used to evaluate the performance of the resulting monoliths.

The best compromise between the different responses was found around 67% PFS and 18% 1,4-butanediol (relative to the total mass), i.e. rather close to the center point. At these conditions, retention times were generally below 15 min and retention factors below 5. Asymmetry values between close to 1 were found, and theoretical plate numbers up to 10,900, which were improvements compared to the central point of the design.

Keywords: Capillary electrochromatography; Monolith; Pore forming solvent; Methacrylate; Pharmaceutical analysis

1. Introduction

In recent years, capillary electrochromatography (CEC) has often been indicated as a new and promising analytical separation technique [1]. CEC is a combination of two separation techniques, i.e. high-pressure liquid chromatography (HPLC) and capillary electrophoresis (CE). The advantages of both techniques are, in principle, gathered in CEC, i.e. high efficiencies due to the use of an electrically driven mobile phase flow, together with high selectivity and sample loading capacity due to the presence of a stationary phase, as in HPLC. The separation of molecules in CEC thus relies on two principles: their partition between the stationary and mobile phases, and their electrophoretic mobilities [2].

Most research in CEC has been done using particle-filled capillary columns [1,3–5]. However, this approach presents some drawbacks. First, frits are required to keep the stationary phase in the column [6]. These frits can cause bubble formation during analysis, and can be responsible for a discontinuity in the stationary phase, potentially resulting in changes of the electrical current [3,4,7]. Furthermore, fabrication of the frits by local heating of the stationary phase, which removes the protective polyimide coating of the capillary, makes the column fragile. Another problem with frits is that particle migration towards the end frit can occur, creating voids in the packing. Finally, certain analytes can show interaction with the frits, which increases their retention [1,8].

A second drawback of particle-based CEC columns lies in the type of particles used. Classically, they are the same as in HPLC, most being silica-based. However, in CEC it is necessary to have a charged stationary phase which enables the generation of an electro-osmotic flow (EOF). Therefore, the acidity of the

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mobile phase is limited, as silanol groups are uncharged at a too low pH [6]. Too high pH values cannot be used either due to the silica degradation above pH 8. Hence, the pH values at which silica-based particles can be used present a possible restriction for the choice of buffer.

To resolve the above-mentioned drawbacks, inherent to CEC analysis with silica particle-based columns, the use of polymer-based monolithic capillary columns was investigated as a possible alternative.

Organic polymer-based monolithic columns have been successfully used in HPLC [9] and therefore it is interesting to investigate their use in CEC. Different kinds of monoliths exist. On one hand, they originate from inorganic components, e.g. silica-based monoliths, while, on the other hand, organic, i.e. polymer-based, monoliths exist. In this study the latter, more specifically methacrylate-based columns, were investigated.

Monolithic columns are prepared in situ by a polymerization reaction and bonded to the capillary walls, eliminating the necessity of frits. Other possible advantages of these columns are the efficiency of separations, the speed of analyses and the possibility of in-house preparation from a simple one-pot reaction [10]. Furthermore, properties like surface functionality and porosity can easily be adapted by changing the composition of the reaction mixture [1,2,11]. The monoliths can be used within a broad pH-range [7,11,12] in which they are chemically stable and remain charged to permit generation of EOF.

A typical polymerization mixture for a polymer-based monolithic column consists of one or more monomers, cross linkers and pore-forming solvents [1]. The polymerization reaction links the monomer molecules to form a polymer chain. Adding the cross linkers creates three-dimensional networks of polymer chains. Pore-forming solvents make this network permeable, so the mobile phase can flow trough.

The pore size distribution is typically bimodal for monoliths [13]. Both smaller and larger pores are formed during the polymerization step. This bimodal pore structure has two advantages. Due to the large pores, the head pressure, or difference in pressure between column inlet and outlet, is smaller than in traditional packed columns [1,13]. The smaller pores, on the other hand, provide the column with a large number of interaction sites.

In CEC, a charged monomer should be incorporated, since a charge on the stationary phase is necessary to generate the EOF [14,15]. To start the polymerization reaction, an initiator is added, which is activated by heat or UV light.

The properties of methacrylate-based CEC columns have been discussed by Eeltink et al. [16]. From experiments comparing packed columns with methacrylate-based monoliths, the best performance was seen with the latter. The number of theoretical plates was comparable. The polymerization reaction was induced by the thermal initiator α,α′-azoisobutyronitrile (AIBN), which Holdšvendová et al. [17] found best in terms of reproducibility.

Previous experiments [18] on this type of columns involved the testing of their applicability for pharmaceutical analysis using a large test set of drug molecules and different mobile phases. The conclusion of these experiments was that further optimization of the stationary phase could possibly result in columns better suited for pharmaceutical analysis.

The improvement of the polymerization-mixture composition is therefore the aim of the current study. This was done by examining the impact of different compositions of polymerization mixtures (specified following an experimental design approach) on the electrochromatographic properties of the resulting columns.

2. Experimental

2.1. Buffers and solutions

Two electrolytes were used in these experiments: a 50 mM ammoniumformate buffer at pH 3, and a 5 mM phosphate buffer at pH 11.5. The mobile phases containing these electrolytes are further referred to as acidic (MPA) and basic (MPB), respectively. Stock solutions of the electrolytes were prepared by dissolving 50 mM formic acid or 5 mM disodium phosphate H_2O (both Merck, Darmstadt, Germany) in milli-Q water. Both were brought to the required pH by adding 25% (v/v) ammonia solution (Merck). Mobile phases were prepared by mixing the electrolyte with ACN (HPLC-grade, Fisher Scientific, Loughborough, UK) in equal volumes. The mobile phases were subsequently degassed on an ultrasonic bath during 15 min and finally filtered through a 0.2 μm FP-Vericel^{TM} membrane filter (Pall, Zaventem, Belgium).

The dead-time marker used in the experiments was acetone (Merck) in buffer. Due to the volatile character of the dead time marker, a 20/80 v/v solution was prepared ex tempore by diluting acetone in the appropriate buffer. The test set consisted of warfarin, ketoprofen, praziquantel, paracetamol (Sigma, St. Louis, MO), metoprolol (Ciba-Geigy, Groot-Bijgaarden, Belgium), pyrene (Aldrich Chemie, Steinheim, Germany) and oxazepam (gift from unknown origin). All samples were dissolved in 50/50 (v/v) water/acetonitrile in a 0.5 mg/ml concentration. For pyrene 30/70 (v/v) water/ACN was used as solvent.

2.2. Capillary electrochromatography

The CEC analyses were performed on a P/ACE^{TM} MDQ Capillary Electrophoresis System (Beckman Coulter, Fullerton, CA). The instrument was controlled by the Beckman 32 Karat software version 4.01 (1999–2000 Beckman-Coulter). Injections were done electrokinetically at −5 kV during 10 s. On-column detection was performed with a diode array UV detector. Chromatograms were recorded at 254 nm for acetone and pyrene, and at 214 nm for all other substances. Analyses were performed at −15 kV. The analyses were conducted in reversed polarity (meaning that the EOF moves from cathode to anode) because of the positive charge of the stationary phase. During analysis a pressure of 4.8 bar was applied on both vials. The temperature of the column was set at 25 °C.

2.3. Preparation of the columns

Methacrylate-based monoliths were prepared in situ in surface treated fused-silica capillaries with an internal diameter of 100 μm and an external diameter of 375 μm (Composite Metal Services, Ilkley, UK). Every column required by the design was made in duplicate. The synthesis is based on the procedure described in [19]. The used monomers were butyl methacrylate (BMA), ethylene dimethacrylate (EDMA) and 2-methacryloyloxyethyltrimethylammoniumchloride (META) (Sigma–Aldrich Chemie, Steinheim, Germany). EDMA has a cross-linking function, whereas META carries a positive charge, necessary for the generation of EOF in the column. α,α′-azoisobutyronitrile (AIBN) (Fluka Chemie, Buchs, Switzerland) was added to initiate the polymerization reaction. The pore-forming solvents were 1-propanol (Merck), 1,4-butanediol (Sigma–Aldrich Chemie) and water (Milli-Q 15 Water purification system, Millipore, Bedford, MA, USA). The polymerization mixture at center point conditions contains 70% (m/m) of PFS of which 30% (m/m) is 1,4-butanediol and 62.57% (m/m) is 1-propanol. The mixture contains 30% (m/m) of monomers with a fixed ratio between EDMA and BMA (2/3), as well as a fixed amount of META diluted in H_2O, i.e. 7.43% (w/w) of the total mixture. The META is added as a 10% (m/m) solution in water. The amounts of PFS and 1,4-butanediol for the other design conditions are specified in Table 1.

The required amounts of each monomer and pore-forming solvent were weighed and mixed. The mixture was then ultrasonicated and degassed by purging with nitrogen (Air liquide, Liège, Belgium), both during 15 min. Finally, it was inserted into a capillary by means of a syringe (500 μl gastight #1750, Hamilton, Bonaduz, Switzerland). The part of the capillary filled with the mixture was 21 cm, and the total capillary length was 31.2 cm. The capillary was sealed with two septa and placed in an oven at 70 °C during 20 h. After polymerization the residual pore forming solvents (PFS) were washed out of the capillary with HPLC-grade methanol (Fisher Scientific) using a flow-splitted L-6000 HPLC pump (Merck-Hitachi, Tokyo, Japan). The flow rate was adapted so that the pressure on the column was around 180 bar. The rinsing step was continued during 1 h after the first elution of liquid.

Table 1
Two-factor central composite design: factor levels and resulting experimental conditions

Experiment	Factor levels		Experimental conditions	
	PFS	1,4-butanediol	%PFS	%1,4-butanediol
1	−1	−1	65	25
2	+1	−1	75	25
3	−1	+1	65	35
4	+1	+1	75	35
5	−1.414	0	63	30
6	+1.414	0	77	30
7	0	−1.414	70	23
8	0	+1.414	70	37
9	0	0	70	30

The %PFS is expressed relative to the total mixture (m/m) and %1,4-butanediol relative to the total PFS (m/m).

A detection window was burned immediately behind the monolithic bed using a capillary burner (Electro-Kinetic Technologies, Broxburn, Scotland). Before analysis each column was preconditioned to obtain a stable current, using the acidic mobile phase. This was done by applying a voltage that increased stepwise (−5, −10, −15, −20 and −25 kV) every 10 min. Afterwards, the dead time marker was injected, until a constant retention time was obtained (usually after three to six injections). Then the test set was injected. When a new mobile phase was used the column was first rinsed with 50/50 (v/v) acetonitrile (ACN)/water during 45 min, followed by conditioning with the mobile phase during 1 h.

2.4. Experimental design and calculations

A setup based on a central composite design was executed to investigate the effect of variations in the polymerization mixture on the retention behavior of the resulting monolithic CEC columns. Varying the ratio of the components of the polymerization mixture generates columns with different properties (e.g. porosity), from which different retention performances are expected. In this case, the total PFS fraction and the concentration of 1,4-butanediol within the PFS were varied. The selected levels are given in Table 1.

Central composite designs examine factors, which usually are defined independent of each other. However, here, 1,4-butanediol (the second factor) is part of the pore-forming solvents (the first factor), and thus the two factors are dependent. As a consequence, the classical symmetric central composite design domain is twisted when both factors are expressed relative to the total mixture mass, as can be seen in Fig. 1. The center point of the domain was chosen as a compromise after consulting different literature sources [2,10,18–20]. It consisted of 70% PFS, of which 30% is 1,4–butanediol. Every experiment was duplicated, thus 18 columns were synthesized.

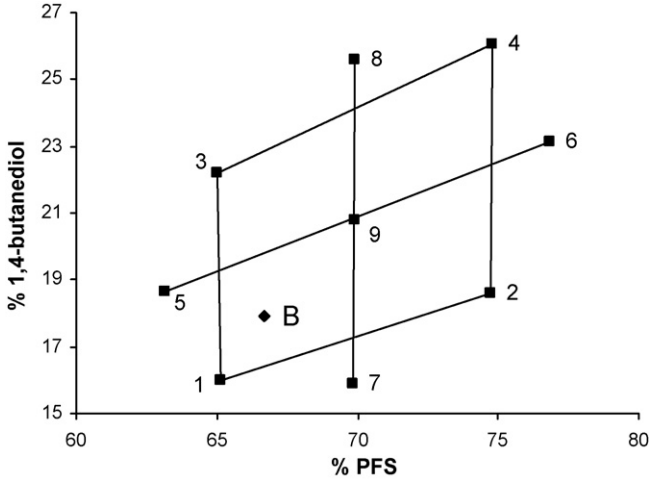

Fig. 1. The examined experimental domain. %PFS and %1,4-butanediol are expressed relative to the total mass. B is the region of best polymerization mixture composition.

A test set of seven molecules, mainly pharmaceutical compounds, differing in hydrophobic properties and pK_a values, was used to test the stationary phases. Four responses were measured for each test compound. From the results measured in the different experiments a quadratic polynomial model was built for each response to estimate their response surfaces [21]. Contour plots, drawn by the Matlab software (version 7.1, 2005, The MathWorks, Natick, MA), based on the above-mentioned models permit to assess the influence of variations in the stationary phase composition on the chromatographic performance.

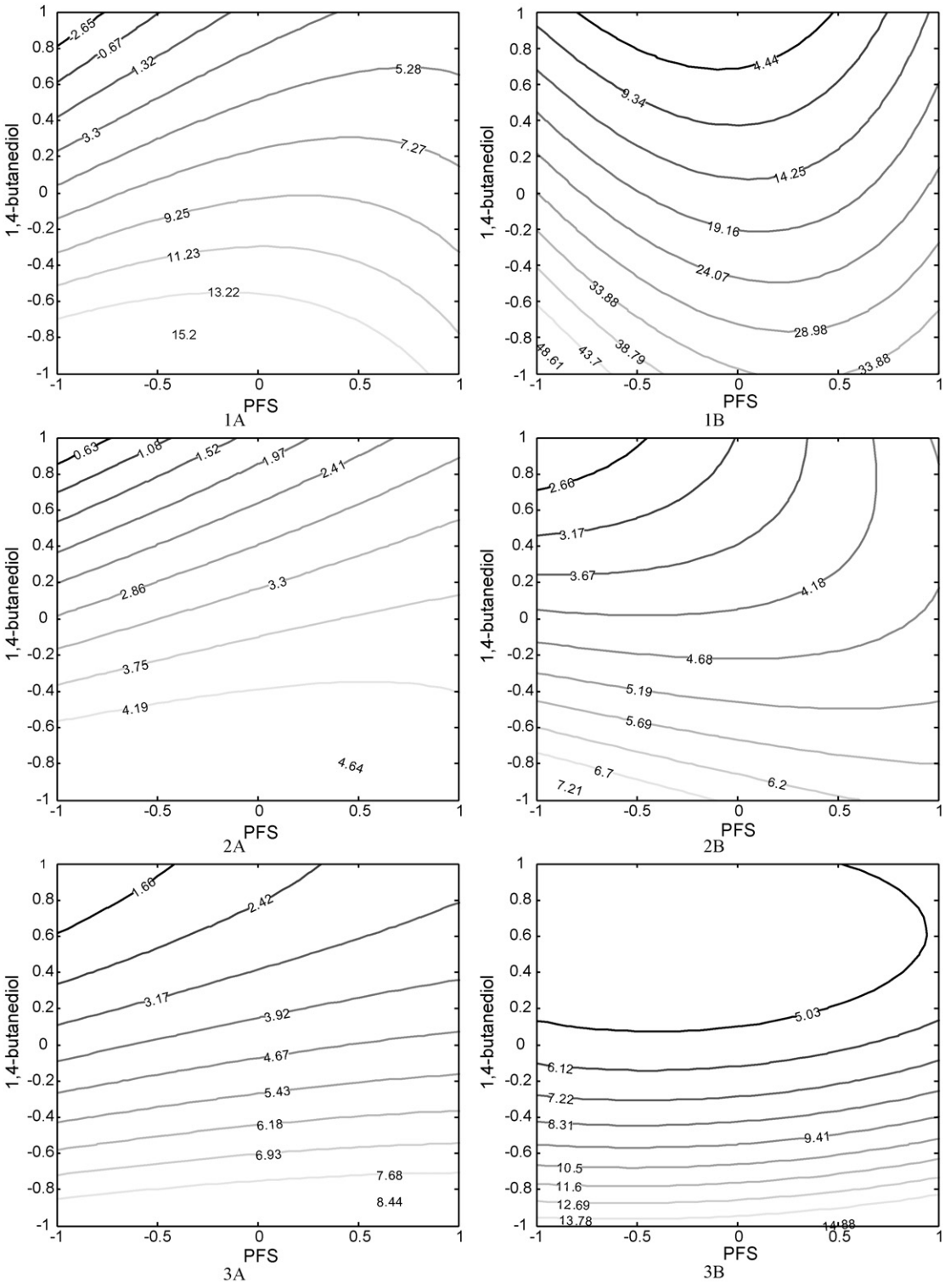

Fig. 2. Contour plots of the retention times of some compounds as a function of the PFS and 1,4-butanediol concentrations in the polymerization mixture. (1) ketoprofen, (2) paracetamol, and (3) praziquantel; (A) MP A and (B) MP B.

2.5. Responses

The evaluated responses were retention time, peak asymmetry and number of theoretical plates, all determined with the Beckman 32 Karat software, and retention factor, calculated with Excel (Microsoft® Excel, 2002). The peak asymmetry and the theoretical plate number are both calculated according to the United States Pharmacopeia [22].

3. Results and discussion

To evaluate the effects of the variations in the polymerization mixture composition on the resulting stationary phases, two approaches were used. The first is a visual evaluation via scanning electron microscope (SEM) photography. The second was based on the chromatographic responses measured, which were modeled and visualized through contour plots.

3.1. General observations

A first observation is that some molecules showed a better retention behavior with one mobile phase. With the acidic mobile phase the basic compound metoprolol was not detected and with basic mobile phase the acid warfarin was not seen. The explanation can be found in the acidic or basic character of these compounds. Acidic molecules are uncharged in the acidic mobile phase and carry a negative charge in the basic mobile phase. In both cases, migration from the cathode towards the anode is expected. Yet, warfarin did not elute at high pH. This can be caused by repulsion from the cathodic injection end, as both the cathode and warfarin are negatively charged. Another possible explanation is a too high interaction of warfarin with the positively charged stationary phase. The acidic compounds warfarin, ketoprofen and paracetamol are therefore preferably analyzed in acidic mobile phase.

Basic compounds, on the other hand, are positively charged at low pH. Possibly they are not injected into the capillary because they are attracted by the cathode or they do not migrate towards the anode because their electrophoretic mobility towards the cathode is higher than the electro-osmotic flow. In a basic mobile phase, they are mainly uncharged and thus elute. For basic compounds the mobile phase is thus preferably basic.

In the next paragraphs, the influence of the two examined factors on the four responses will be discussed. From these results the stationary phase composition displaying a good compromise for retention, peak asymmetry and efficiency will be derived.

3.2. Retention time and selectivity

The contour plots generated from the retention-time modeling (Fig. 2) show that an increase in the ratio 1,4-butanediol/1-propanol within the PFS causes a decrease of retention times. This is caused by the higher polarity of 1,4-butanediol compared to 1-propanol. At higher 1,4-butanediol concentrations the polarity of the PFS-mixture is therefore higher. Due to this, the onset of the phase separation in the polymerization process occurs early, permitting the monomers to diffuse further away from each other, resulting in the formation of larger macropores [10].

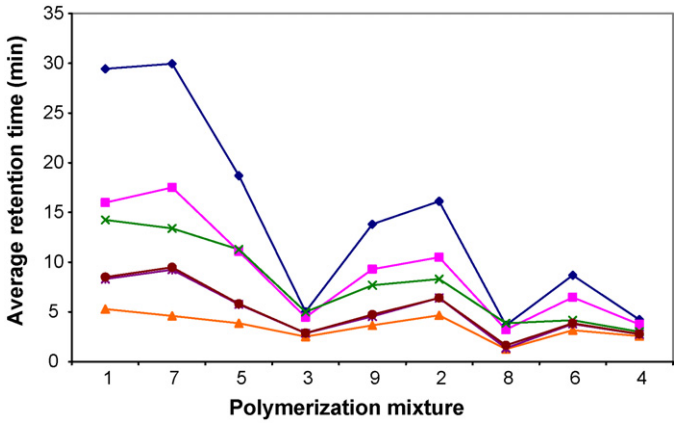

Fig. 3. Retention times at the different stationary phase compositions (see Fig. 1) with MP A: (♦) warfarin; (■) ketoprofen; (▲) paracetamol; (×) pyrene (*tR*/10); (*) praziquantel; (●) oxazepam.

Similar results were obtained by Grafnetter et al. [23], who described how low 1,4-butanediol concentrations result in smaller macropores and larger mesopores. The presence of large mesopores, and thus of a high specific surface, results in a larger number of interaction sites [13]. The size of the macropores, on the other hand, influences the speed of the eluent flow and therefore the speed of the analysis.

Fig. 3, which represents the retention times in relation to the polymerization mixture (see Table 1), confirms that the highest retention times are indeed obtained on the columns with the lowest 1,4-butanediol levels (mixtures 1 and 7 mainly, less on 2 and 5).

Conclusions concerning the selectivity of the different phases can also be drawn from Fig. 3. In comparison to the center

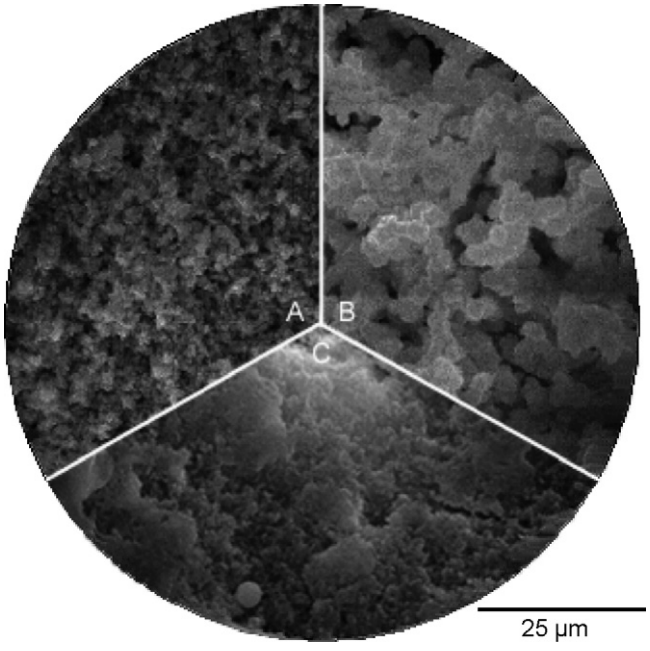

Fig. 4. SEM pictures of three stationary phases: (A), (B) and (C) represent phases 9, 4 and 1, respectively.

point (9), phases 1, 7, 5 and 2 display larger selectivity differences for the tested compounds. This is accompanied by a larger increase in retention times for most compounds on phases 1 and 7.

Generally, as the PFS-level increases, at constant 1,4-butanediol level, the retention time is rather constant, as can be seen in Fig. 2. This means that the amount of PFS has little influence on the retention times observed. The 1,4-butanediol

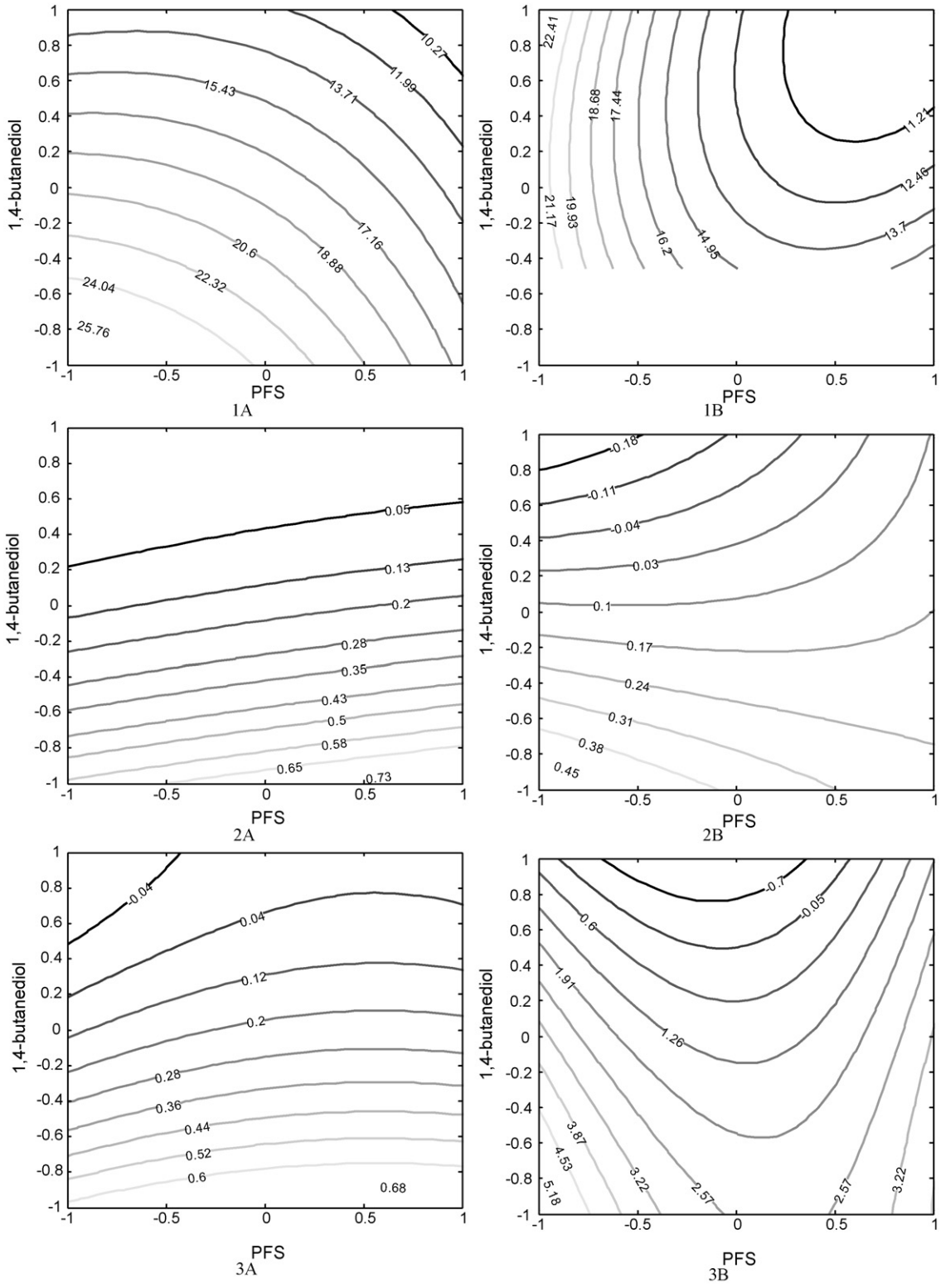

Fig. 5. Contour plot of the retention factors of some compounds as a function of the PFS and 1,4-butanediol concentrations in the polymerization mixture. (1) Pyrene, (2) praziquantel, and (3) oxazepam; (A) MP A and (B) MP B. The contour plot of pyrene in basic mobile phase is incomplete because there was no elution of pyrene within 120 min at the lowest 1,4-butanediol levels.

fraction or, in other words, the within-PFS composition is more important.

The experimental results and the theoretical expectations concerning the influence of the polymerization mixture composition on the pore size concur with the scanning electron microscope pictures (Fig. 4). Stationary phase compositions 9 (A), 4 (B) and 1 (C) are displayed clockwise. These correspond to the center point of the setup, the highest, and the lowest 1,4-butanediol levels, respectively (Fig. 1). It can be seen that as 1,4-butanediol levels increase, the size of the macropores and the inter-pore globules also do.

Another observation that could be made is that the retention time is higher in the basic mobile phase than in the acidic (Fig. 2). Since the pH of the mobile phase has no influence on the charge of the stationary phase, the explanation has to be searched for in both the higher ionic strength of the basic mobile phase and the charge of the compound. An increased ionic strength indeed results in a lower EOF [6]. The dissociation might also play a role for ketoprofen and paracetamol in Fig. 2.

3.3. Retention factor

The retention factor (k) is expected to follow the trends of the retention time, as the former is calculated from the latter. However, the retention factor provides supplementary information by relating the retention time to the dead time. In this way, the retention of some tested substances could be put into perspective. For example, paracetamol elutes quickly, which results in favorable retention-time surface plots. The retention-factor surface plot however provides another view: paracetamol is not retained and even elutes shortly before the dead-time marker, resulting in negative k values. Small or negative k values are not desired because this means there is little or no interaction of the analyte with the stationary phase. Pyrene on the other hand always elutes with relatively high k values, indicating a major interaction with the stationary phase due to a reverse-phase interaction mechanism.

Even though the retention times tend to indicate retention on the stationary phase (e.g. praziquantel 5–14 min), there was hardly any interaction with the stationary phases, as already discussed for paracetamol. This can, for instance, also be seen for praziquantel and oxazepam (Fig. 5). A possible reason could be a too high solvent strength of the applied mobile phase.

Although there is hardly any retention, the contour plots for the retention factor of praziquantel and oxazepam show a similar trend as the retention time plots. For oxazepam at basic conditions retention is observed on the columns synthesized with a low 1,4-butanediol content. Compared to the acidic mobile phase conditions this will be due to a reduced charge of oxazepam. The behavior of the oxazepam retention is similar to what was discussed earlier, i.e. retention is mainly affected by the 1,4-butanediol content in the polymerization mixture, while the total PFS content is less important.

The fact that the EOF is lower in the basic mobile phase (see section 3.2) results in a higher dead time. This explains why substances showing a higher retention time in basic than in acidic mobile phase, e.g. paracetamol and praziquantel (Fig. 2), display little interaction (i.e. k values close to zero) in the retention factor contour plots.

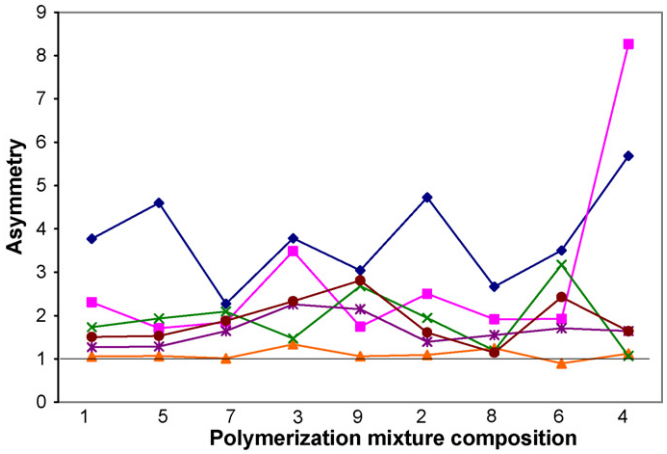

Fig. 6. The asymmetry at the different stationary phase compositions with MP A. (♦) Warfarin; (■) ketoprofen; (▲) paracetamol; (×) pyrene; (*) praziquantel; (●) oxazepam.

3.4. Asymmetry factor

Some remarks can be made concerning the peak asymmetry (As). First, some molecules have a high asymmetry at all points of the design. The peak asymmetry of the test compounds can be observed in Fig. 6. The most retained compounds pyrene and warfarin also have the highest peak asymmetry. As the average k decreases over ketoprofen, oxazepam, praziquantel and paracetamol, the average As does too. It is logical that hardly retained peaks are still symmetric when they elute.

The contour plots in Fig. 7 show that As often is best or good for stationary phases prepared with low PFS and 1,4-butanediol concentrations. For some compounds other points of the domain (e.g. high PFS and low 1,4-butanediol) may give the best As but these conditions have less good retention properties.

3.5. Theoretical plate number

The contour plots reveal that the theoretical plate number (N) generally is the highest at low 1,4-butanediol levels (Fig. 8). This is also the area with the highest retention, especially when the PFS concentrations is also low, so the plate number of a test compound peak is proportional to the retention of that compound on the column.

The number of theoretical plates generally decreases slightly as the percentage of PFS decreases. However, as for retention, the influence of the PFS concentration is considerably less than that of the 1,4-butanediol content.

The overall number of theoretical plates is not as high as one may expect. Some experiments did not result in peaks with more than 5000 theoretical plates per meter. These relatively low plate numbers concur with the findings of other groups [24].

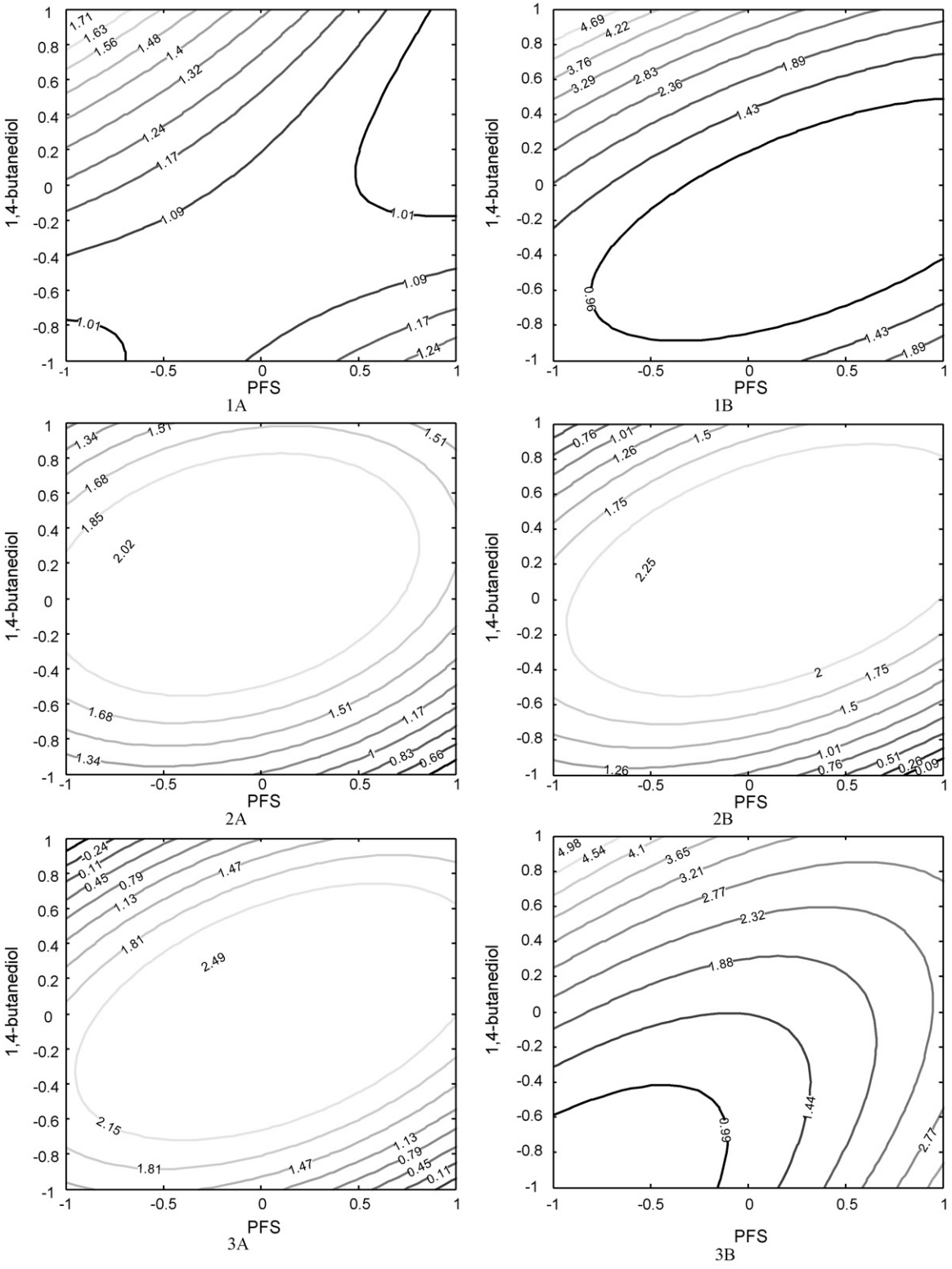

Fig. 7. Contour plot of the peak asymmetry of some compounds as a function of the PFS and 1,4-butanediol concentrations in the polymerization mixture. (1) Paracetamol, (2) praziquantel, and (3) oxazepam; (A) MP A and (B) MP B.

3.6. Derringer's desirability functions

Simultaneous assessment of all responses is very important to find a stationary phase composition with an acceptable retention behavior. To solve this multicriterion decision-making problem Derringer's desirability functions were used [21]. These functions transform the measured responses to a dimensionless desirability scale between 0 and 1, so that values of several responses can be combined.

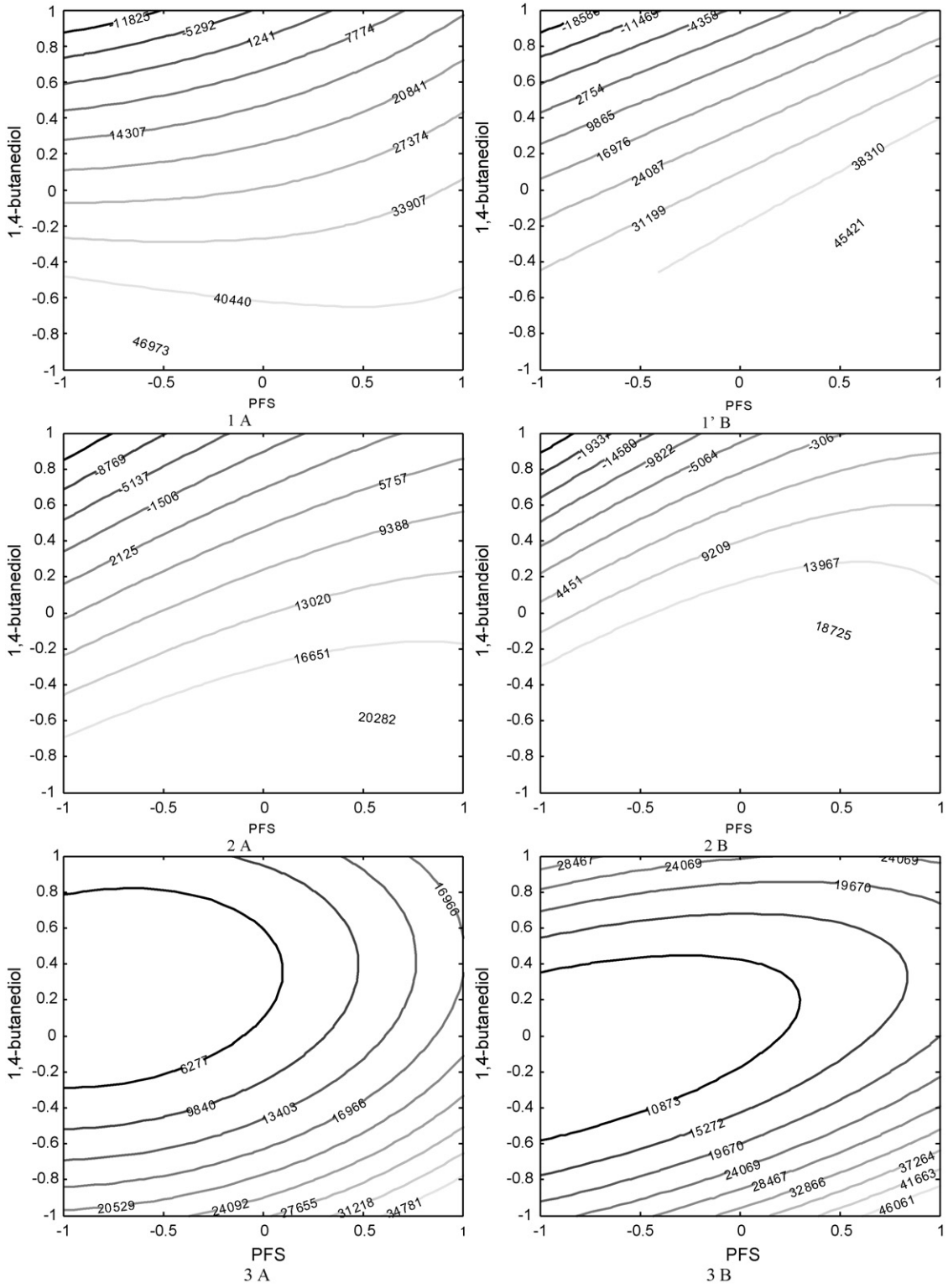

Fig. 8. Contour plot of the plate number (N/m) of some compounds as a function of the PFS and 1,4-butanediol concentrations in the polymerization mixture. (1) Paracetamol, (1′) metoprolol, (2) pyrene, and (3) praziquantel; (A) MP A and (B) MP B.

A desirability value, d_1, d_2 and d_3, was calculated for each test compound for the responses k, As and N, respectively. The most desired result for a response was given desirability 1 and an unacceptable value for a response was given the desirability 0. Intermediate values are calculated according to a transformation function. This function is different depending on what is considered best, e.g. for k and N larger values are preferred, while for As a value closer to 1 is preferred. Linear transformation functions were applied to obtain the desirability values d_k, d_{As} and d_N. The three desirabilities of one

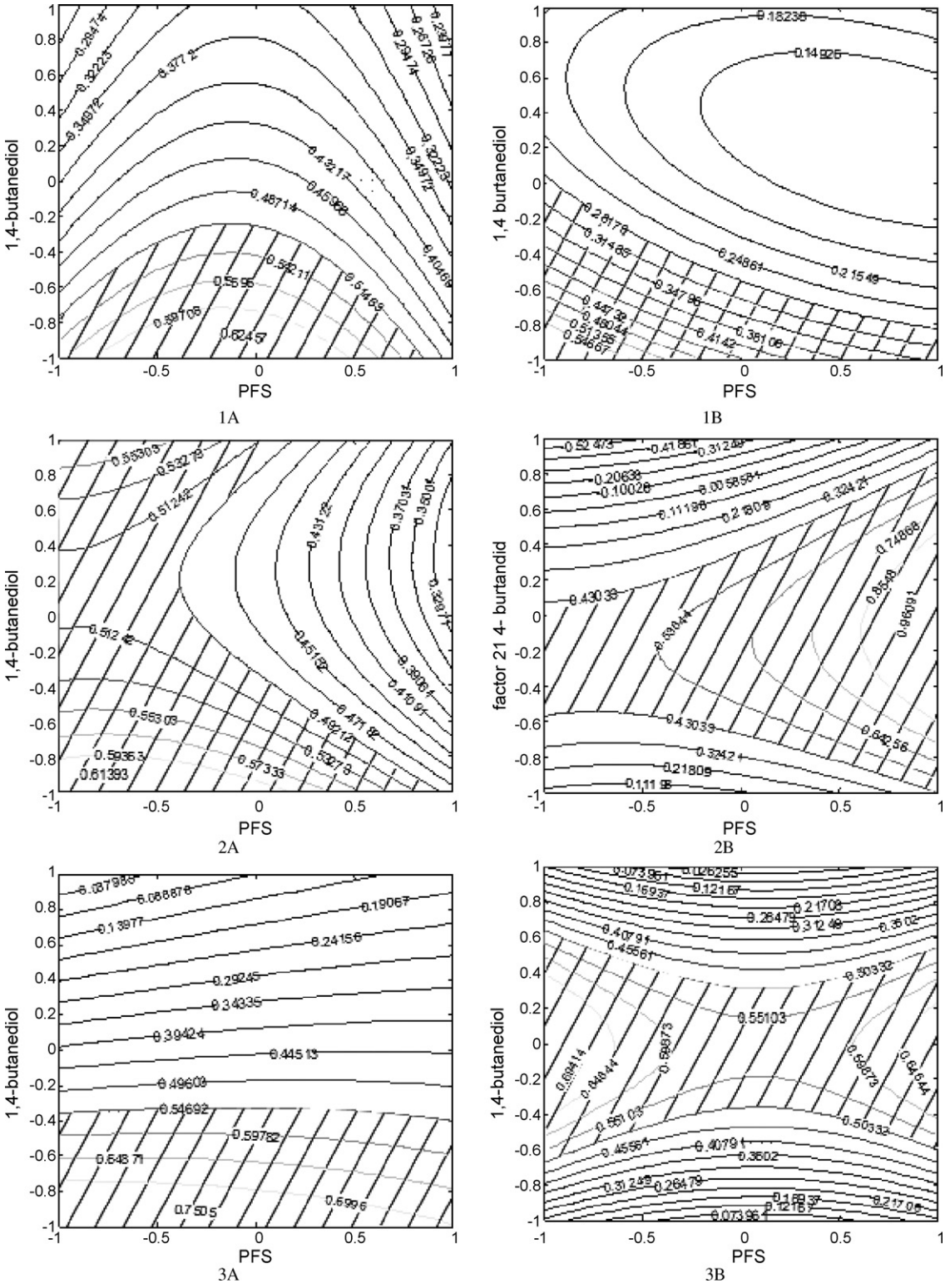

Fig. 9. Contour plot of the global Derringer's desirability values of some compounds as a function of the PFS and 1,4-butanediol concentrations in the polymerization mixture. (1) Ketoprofen, (2) warfarin, (2′) metoprolol, and (3) pyrene; (A) MP A and (B) MP B. The shaded areas represent the higher Derringer's desirability values.

molecule on one stationary phase are then multiplied and finally the cube root of this product is calculated. The obtained number is called the global Derringer's desirability value. The advantage of multiplying the desirability values is that if one of the responses has an unacceptable value, then the global Derringer's desirability value D will also be unacceptable. The response D can then be modeled and its contourplots drawn (Fig. 9).

The stationary phase compositions where the Derringer's desirability value is higher than the center point, or the regions

where D is best, can then be found (Fig. 9). The regions where D was highest are shaded in Fig. 9.

It is immediately clear that it is difficult to find a composition of the polymerization mixture where all requirements are fulfilled, i.e. where D is high for all substances. Compromises have to be made to obtain a column with a satisfying retention behavior.

The most favorable chromatographic performance is a rather low retention time, but a high selectivity, with peak asymmetry values approaching 1 and a high efficiency. Based on these principles the individual contour plots were assessed, as well as the Derringer desirability value plots.

The areas of polymerization mixture composition showing favorable response values were located, and a polymerization

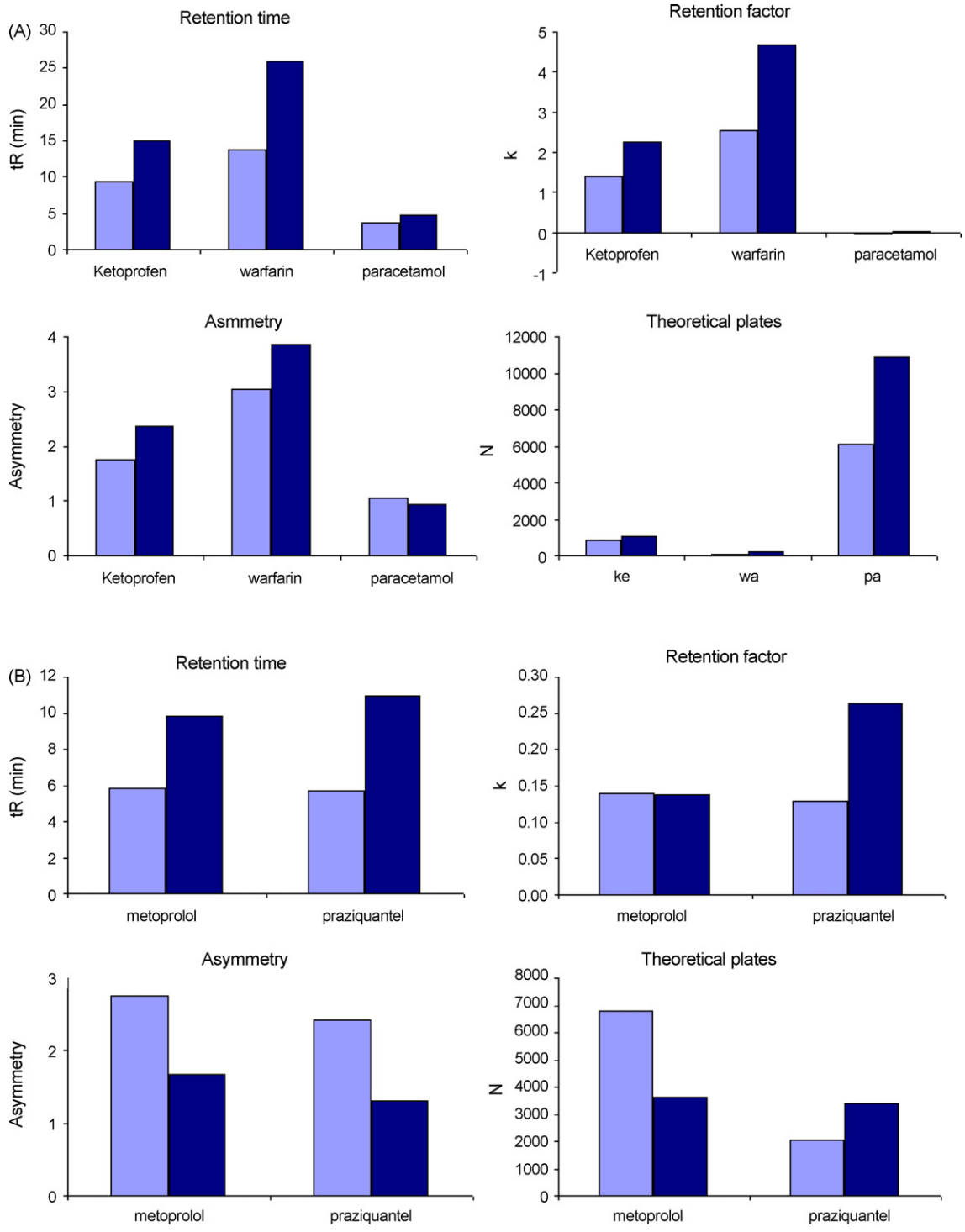

Fig. 10. Comparison of the responses obtained on the most favorable column (black) and the central point column (grey). (A) Acidic molecules in acidic mobile phase, (B) basic and neutral molecules in basic mobile phase, and (C) neutral and basic molecules in acidic mobile phase.

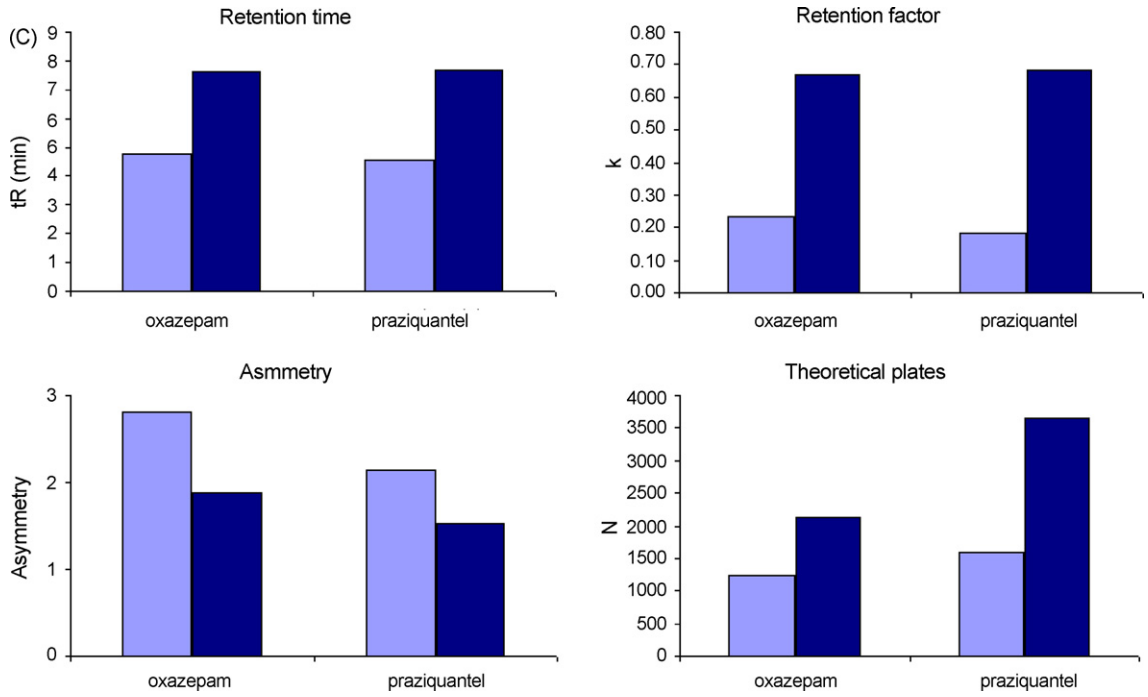

Fig. 10. (*Continued*).

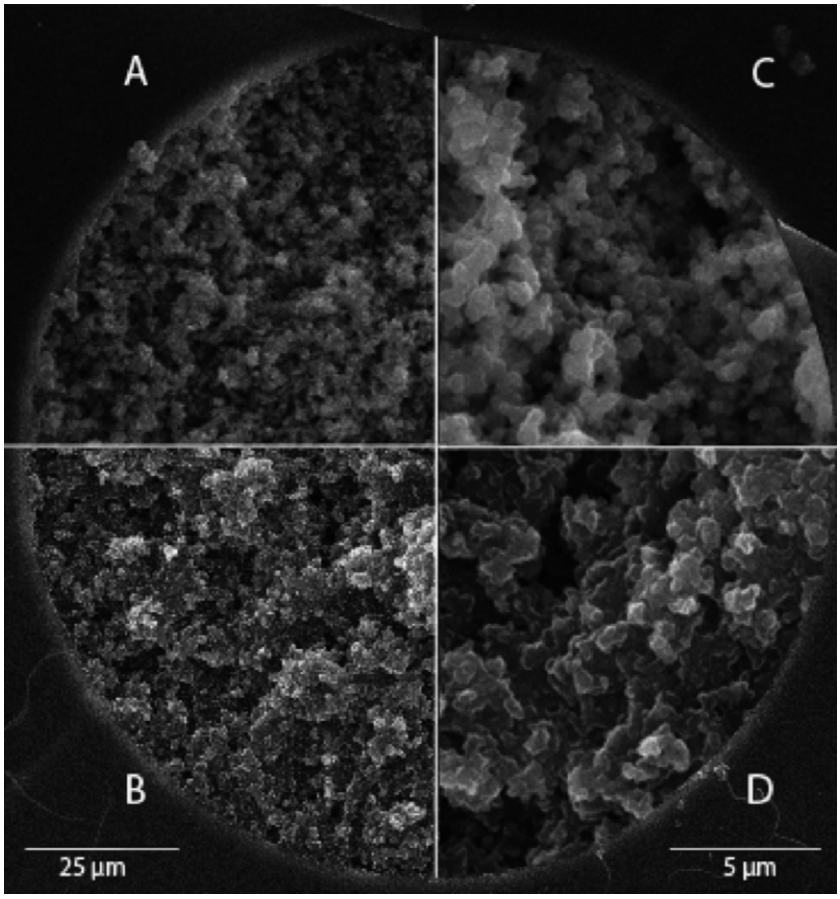

Fig. 11. SEM pictures of the central point of the design ((A) and (C) at different scales) and the best-compromise stationary phase ((B) and (D) at different scales).

mixture composition that seems a good compromise for all responses was identified. Such polymerization mixture is situated around 67% PFS and 18% 1,4-butanediol, both expressed relative to the total polymerization mixture mass (B in Fig. 1).

4. Testing the best stationary phase

Finally the selected stationary phase composition was synthesized and tested by injecting all molecules in the appropriate mobile phase. The comparison was made with the center point columns. The acidic molecules were tested in the mobile phase with pH 3. The results are shown in Fig. 10A. Retention times of ketoprofen and paracetamol were below 15 min, and warfarin eluted at 26 min. The retention factors of ketoprofen and warfarin were between 1 and 5. Paracetamol displayed a k of 0.031, i.e. it is not retained on either stationary phase. The asymmetry and the number of theoretical plates of the acidic compounds increased.

Metoprolol was tested at basic mobile phase (Fig. 10B). The retention time increased, nevertheless remaining below 10 min. A good asymmetry was seen with the best stationary phase, but the number of theoretical plates decreased. The retention factor was equivalent to the one obtained on the center point column.

Oxazepam was tested at both pH values. However, at high pH, it did not elute. At low pH, the retention time of oxazepam increased slightly (Fig. 10C). The retention factors, the asymmetry and the number of theoretical plates improved as well.

Both neutral compounds (pyrene and praziquantel) were tested at both pH values. Pyrene, however, did not elute within 200 min on these columns. This was expected, as earlier it did not elute earlier either at stationary phase compositions with low 1,4-butanediol-levels. Praziquantel eluted with both mobile phases (Fig. 10B and C). Both the retention times and retention factors increased in both mobile phases but k remained below 1. The plate numbers increased at both pH's, and the asymmetry values decreased. Both mobile phases give comparable results for praziquantel.

For a visual evaluation of the best-compromise stationary phase, SEM pictures were taken (Fig. 11). Its structure resembles that of the central point, which is expected since their composition is rather similar. The difference in pore structure and cluster size can especially be noticed when comparing with the SEM pictures of the other stationary phases (Fig. 4).

5. Conclusion

Although monolithic columns can be an alternative to particle-filled CEC columns for pharmaceutical analysis, earlier experiments indicated optimization of the composition of the polymerization mixture may be favorable. Stationary phases made from different polymerization mixture compositions were studied for their chromatographic performance for drug molecules.

Modeling the experimental results allowed finding a composition around 67% PFS and 18% 1,4-butanediol (both expressed relative to the total amount) in which the responses corresponded best to general desirabilities.

This stationary phase composition was tested, resulting in retention times generally below 15 min. The retention factors were higher at this stationary phase than at the center point, signifying an increased selectivity. Moreover, k remained below 5, which is preferable. Occasionally it is too low, and might be improved using a mobile phase with a lower solvent strength. Compared to the center point stationary phase, the asymmetry values were closer to 1 and the theoretical plate numbers increased.

The conclusion of the above study is that methacrylate-based monolithic stationary phases can be useful in the analysis of drug molecules using capillary electrochromatography as separation technique.

In future experiments, variations of the mobile phase composition should be tested. The influence of the solvent strength and the pH and the use of other organic modifiers are factors to be examined during the optimization of the separation of a given mixture.

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References

[1] C. Legido-Quigley, N.D. Marlin, V. Melin, A. Manz, N.W. Smith, Electrophoresis 24 (2003) 917–944.
[2] Á. Végvári, A. Guttman, Electrophoresis 27 (2006) 716–725.
[3] M.G. Cikalo, K.D. Bartle, M.M. Robson, P. Myers, M.R. Euerby, Analyst 123 (1998) 87R–102R.
[4] L.A. Colón, G. Burgos, T.D. Maloney, J.M. Cintrón, R.L. Rodríguez, Electrophoresis 21 (2000) 3965–3993.
[5] N.W. Smith, A.S. Carter-Finch, J. Chromatogr. A 892 (2000) 219–255.
[6] I.S. Krull, R.L. Stevenson, K. Mistry, Capillary Electrochromatography and Pressurized Flow Capillary Electrochromatography: An Introduction, HNB Publishing, New York, 2000.
[7] F. Svec, E.C. Peters, D. Sýkora, J.M.J. Fréchet, J. Chromatogr. A 887 (2000) 3–29.
[8] B. Behnke, J. Johansson, S. Zhanga, E. Bayera, S. Nilsson, J. Chromatogr. A 818 (1998) 257–259.
[9] F. Svec, J. Sep. Sci. 27 (2004) 747–766.
[10] S. Eeltink, J.M. Herrero-Martinez, G.P. Rozing, P.J. Schoenmakers, W.T. Kok, Anal. Chem. 77 (2005) 7342–7347.
[11] E.F. Hilder, F. Svec, J.M.J. Fréchet, Electrophoresis 23 (2002) 3934–3953.
[12] E.F. Hilder, F. Svec, J.M.J. Fréchet, J. Chromatogr. A 1044 (2004) 3–22.
[13] M. Merhar, A. Podgornik, M. Barut, M. Žigon, A. Štrancar, J. Sep. Sci. 26 (2003) 322–330.
[14] G. Ping, L. Zhang, L. Zhang, W. Zhang, P. Schmitt-Kopplin, A. Kettrup, Y. Zhang, J. Chromatogr. A 1035 (2004) 265–270.
[15] F. Svec, J. Sep. Sci. 28 (2005) 729–745.
[16] S. Eeltink, G. Desmet, G. Vivó-Truyols, G.P. Rozing, P.J. Schoenmakers, W.T. Kok, J. Chromatogr. A 1104 (2006) 256–262.
[17] P. Holdšvendová, P. Coufal, J. Suchánková, E. Tesařová, Z. Bosáková, J. Sep. Sci. 26 (2003) 1623–1628.
[18] D. Mangelings, I. Tanret, V. Meert, S. Eeltink, P.J. Schoenmakers, W.Th. Kok, Y. Vander Heyden, J. Chromatogr. Sci. 45 (2007) 578–586.

[19] M. Lämmerhofer, F. Svec, J.M.J. Fréchet, W. Lindner, J. Chromatogr. A 925 (2001) 265–277.
[20] B. Buszewski, M. Szumski, S. Sus, LC–GC Eur. 15 (2002) 792–798.
[21] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997.
[22] United States Pharmacopeia, 25th ed., United States Pharmacopeial Convention, Rockville, MD, 2001.
[23] J. Grafnetter, P. Coufal, E. Tesařová, J. Suchánková, Z. Bosáková, J. Ševčík, J. Chromatogr. A 1049 (2004) 43–49.
[24] S. Eeltink, G. Desmet, G. Vivó-Truyols, G.P. Rozing, P.J. Schoenmakers, W.Th. Kok, J. Chromatogr. A 1104 (2006) 256–262.