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Characterization of calixarene- and resorcinarene-bonded stationary phases
I. Hydrophobic interactions

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Abstract

New HPLC phases with supramolecular selectors on the basis of calixarenes and resorcinarenes were investigated for the first time by means of empirically based test mixtures. The tests, originally developed for common reversed phases, were chosen to evaluate fundamental chromatographic properties of the new materials. In the first part of these studies three descriptors (hydrophobic retention capacity—k'_{hyd}, hydrophobic selectivity—α_{hyd}, steric selectivity—α_{ster}) were determined. Except of higher α_{ster} values and α_{hyd} values with some methods for the resorcinarene phase the phases with supramolecular selectors were classified as less hydrophobic possessing lower hydrophobic and steric selectivities compared to three RP-C_{18} phases and a *p-tert*-butyl phenyl ether phase. The results were confirmed by means of a separation of geometric isomers of thioxanthenes. In contrast, in spite of lower k'_{hyd} and α_{hyd} values calixarene phases were more selective than the Kromasil-C_{18} phase in the separation of gestagenic and androgenic steroids due to specific interactions with the steroids of similar lipophilicity.

Keywords: Stationary phases, LC; Hydrophobic interactions; Calixarene; Resorcinarene; Steroids; Thioxanthenes

1. Introduction

The differences in many commercially available stationary phases are due to differences in their hydrophobic, ionic and polar properties [1]. Until now, there exists no universally accepted chromatographic test to chose an appropriate packing material for a particular separation problem [2]. In reversed-phase chromatography, many descriptors can give certain information to estimate the chromatographic behavior of the stationary phases; i.e. the type of the bonded ligand and its bondage to the surface, the surface coverage, the surface area and the support material are used to explain the differing properties of the chromatographic materials [3]. Nevertheless, the use of empirically based test mixtures is often inevitable because phases behave different than expected by their chemical and physical parameters. Test runs can provide information concerning the hydrophobic (hydrophobic retention capacity, hydrophobic selectivity, steric selectivity) and polar properties (silanol group activity, polar selectivity, ion exchange selectivity, complexation capacity) [4] of a column.

Interest in calixarene- and resorcinarene-bonded stationary phases in HPLC for the separation of positional [5–9] and geometric isomers [5,10–13] and other solutes of pharmaceutical interest [7,14,15] is growing. Even optical isomers were discriminated by specifically modified chiral phases [16,17]. Some selectivities were due to interactions between analytes and cavities formed by the supramolecules. Hence, not only hydrophobic but also more specific interactions are responsible for the higher selectivities for particular analytes on these phases.

It was shown that same methylene and phenylene selectivities can be obtained on achiral calixarene phases as well as on common RP-C_{18} phases with higher carbon content by varying the solvent strength of the mobile phase [14]. To our knowledge, no studies have yet been performed to character-

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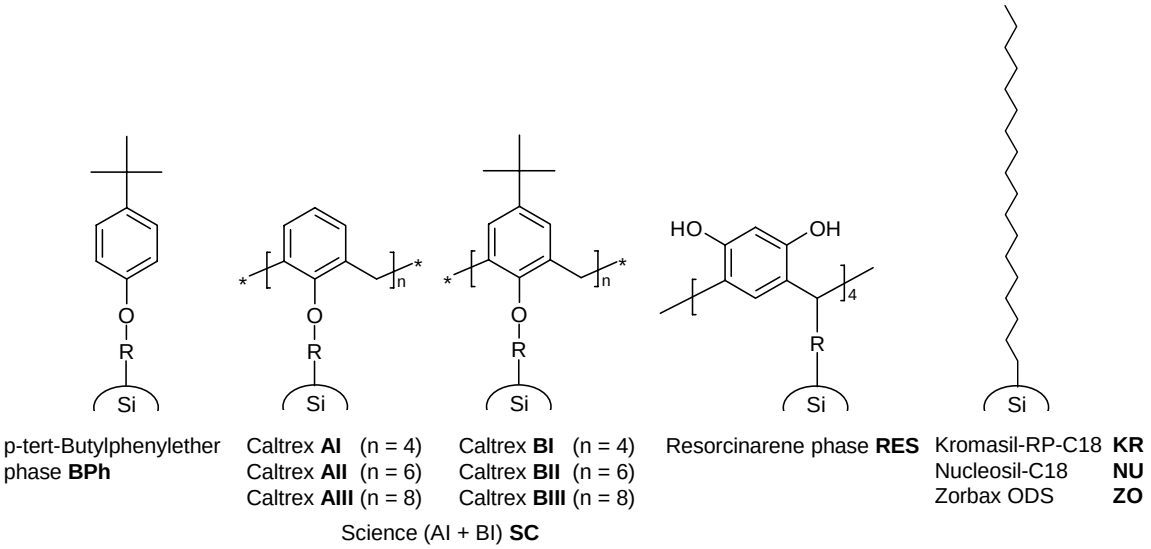

Fig. 1. Chemical structures of bonded selectors of the investigated chromatographic materials.

ize fundamental chromatographic descriptors of calixarene- and resorcinarene-bonded stationary phases. These materials contain supramolecular host molecules bond to silica. Because of the formation of host–guest complexes between solutes and calixarenes or resorcinarenes, respectively, a transfer of known test methods for common reversed phases to these phases would seem difficult. Additionally, problems arise when comparing one defined descriptor on several phases having different test conditions and test mixtures. Hence, the classifications of RP-C_{18} materials depend on the method used [4].

The first part of our studies compares test methods used for the determination of parameters describing the hydrophobic parts of the stationary phases. Fundamental chromatographic properties of calixarene and resorcinarene phases were determined and a comparison with common reversed phases was undertaken. The results were evaluated by separations of (*E*)- and (*Z*)-isomers of thioxanthenes and of neutral steroids used as gestagens and androgens. Investigations of polar interactions of the new phases and a chemometric analysis will be published in the next part of our studies [18].

2. Experimental

2.1. Chemicals

Toluene, naphthalene, acenaphthene, diphenyl and sodium dihydrogene phosphate (NaH_2PO_4) were purchased from Merck KG (Darmstadt, Germany). Butylbenzene, pentylbenzene and triphenylene were obtained from Acros Organics (New Jersey, USA). Ethylbenzene and anthracene were supplied from Berlin Chemie (Berlin, Germany). Benzene was purchased from Riedel-de-Haën (Seelze, Germany) and *o*-terphenyl was obtained from Sigma–Aldrich Chemie (Steinheim, Germany). Norethisterone, norethisterone acetate, chlormadinone acetate and testosterone propionate were kindly supplied by Salutas Pharma GmbH (Barleben, Germany). (*Z*)- and (*E*)-isomers of flupentixol and clopenthixol were obtained from Tropon (Cologne, Germany). All analytes were of the highest quality available.

HPLC grade methanol (MeOH) and acetonitrile (MeCN) were purchased from Applichem (Darmstadt, Germany). Water was obtained by bidistillation.

2.2. Equipment

The separations were performed on a HP1090 II (Hewlett-Packard, Waldbronn, Germany). The model was equipped with a diode array detector.

2.3. Columns

The Caltrex-phases (calixarene phases), the resorcinarene phase (RES) and the *p-tert*-butylphenylether phase (BPh) were supplied from Synaptec (Greifswald, Germany). The calixarene phases contain silica-bonded calixarenes of different ring-size and *p*-substitution (Fig. 1). The ligands were immobilized via hydrophobic spacers on endcapped silica (Kromasil Si 100, 5 μm, specific surface area/BET: 311 m^2/g, pore volume: 0.9 ml/g, manufacturer: EKA Chemicals (Bohus, Sweden). The immobilization procedure is patented (DE 19602393, EP 0786661 A2 and Wo 97/27479).

The Kromasil RP-C_{18} phase (KR) was purchased from CS Chromatography Service (Langerwehe, Germany). The Nucleosil-C_{18} (NU) and the Zorbax-ODS (ZO) phases were obtained from Säulentechnik Knauer GmbH (Berlin, Germany).

All phases have particle diameters of 5 μm. They were of dimensions of 125 mm × 4 mm i.d. with exception of NU and ZO (120 mm × 4 mm i.d.). Columns with dimensions

Table 1
Characteristics of the columns

	Pore size (Å)	Molar surface coverage (μmol/m^2)	Specific surface area (m^2/g)	Carbon content (%)
AI	100	0.95	280	14.0
AII	100	0.32	268	8.8
AIII	100	0.53	282	15.3
SC	100	0.81	275	14.2
BI	100	0.71	275	14.3
BII	100	0.20	264	8.1
BIII	100	0.39	279	15.3
BPh	100	1.34	261	8.1
RES	100	0.54	272	13.5
KR	100	2.94	310	19.0
NU	120	–	200	11.0
ZO	70	–	300	10–12

of 250 mm × 4 mm i.d. were used for the investigations in 3.4. The materials differed concerning pore diameters, surface coverages, specific surface areas and carbon contents (Table 1).

2.4. Chromatography

Chromatographic experiments were performed with isocratic eluents throughout, which were degassed ultrasonically prior to use. The conditions (mobile phases, detector wavelengths, concentrations of analyte samples, injection volumes) were used as suggested by the authors of the respective method without change [19–23]. Only, in the method of Neue et al. [24] NaH_2PO_4 was used instead of K_2HPO_4. The pH value of this buffer was adjusted with H_3PO_4 or NaOH to 7.0 or to 3.5 for the investigations of steroids, respectively.

In all cases, the column temperature was set at 40 °C and the flow rate was 1 ml/min. The hold-up times (t_0) were determined according to Walters et al. [20] from injections of uracil with UV detection at 254 nm in a MeCN/water 65:35 (v/v) mixture as the mobile phase.

3. Results and discussion

Six different calixarene phases and one resorcinarene phase (Fig. 1) were characterized by different test mixtures developed for RP-C_{18} phases. The selectors differ in their ring size and their substitution by *p-tert*-butyl groups at the upper rim of the cavities. Additionally, the "science phase" SC contains a 1:1 mixture of AI and BI. Furthermore, a phase with monomer *p-tert*-butylphenyl as selector was chosen to compare the influence of the supramolecular hosts formed by calixarenes and resorcinarenes, respectively. Also three RP-C_{18} phases with different base silicas were used to evaluate and compare chromatographic parameters obtained on all materials.

Empirical tests were used to give information about hydrophobic (k'_{hyd}—hydrophobic retention capacity, α_{hyd}—hydrophobic selectivity) and steric properties (α_{ster}—steric selectivity) of the columns. The test mixtures were selected in such a way that the test solutes as well as the chromatographic conditions differ as much as possible. Thereby, the generalized conclusions concerning every single descriptor were confirmed by more than one method.

3.1. Hydrophobic retention capacity

Hydrophobic hydrocarbons were used as test solutes for the determination of the hydrophobic retention capacity k'_{hyd} (Table 2). With exception of Neue et al. [24], all authors had employed alkyl substituted benzenes. Nevertheless, acenaphthene is a partially hydrogenated aromatic. Thus, it possesses aromatic as well as alicyclic elements and has certain similarity to the other solutes.

The greatest differences in absolute k'_{hyd} values between the phases were observed with k'_{hyd} of ethylbenzene according to the method of Engelhardt et al. [19] (Fig. 2). In contrast, the k'_{hyd} values of toluene according to the method of Walters et al. [20] did not differ as much. Thus, k'_{hyd} (ethylbenzene) values should give the most distinct results because even differences between phases with similar hydrophobicity were detected. However, it is unfavorable to work with $k'_{hyd} > 30$ because of long analysis times.

A good comparison between the methods can be obtained by standardization of the absolute k'_{hyd} values according to:

$$k'_{standardized} = \frac{k' - \bar{k}'}{\sigma} \quad (1)$$

with $\bar{k}'$ as the mean of all k' and σ as the standard deviation. Thus, it was shown that almost all methods lead to a similar

Table 2
Chromatographic conditions of chosen test methods for the determination of hydrophobic retention capacities k'_{hyd} and hydrophobic selectivities α_{hyd}

Author	Hydrophobic capacity k'_{hyd}	Hydrophobic selectivity α_{hyd}	Mobile phase (v/v)
Engelhardt [19]	k' (toluene)	α (ethylbenzene/toluene)	MeOH/H_2O, 49:51
	k' (ethylbenzene)		MeOH/H_2O, 49:51
Walters [20]	k' (toluene)	α (anthracene/benzene)	MeCN/H_2O, 65:35
Tanaka [21]	k' (pentylbenzene)	α (pentylbenzene/butylbenzene)	MeOH/H_2O, 80:20
Olsen [22]	k' (toluene)	–	MeOH/H_2O, 65:35
Goldberg [23]	–	α (anthracene/naphthalene)	MeOH/H_2O, 85:15
Neue [24]	k' (acenaphthene)	α (acenaphthene/naphthalene)	MeOH/20 mM NaH_2PO_4 (pH = 7.0), 65:35

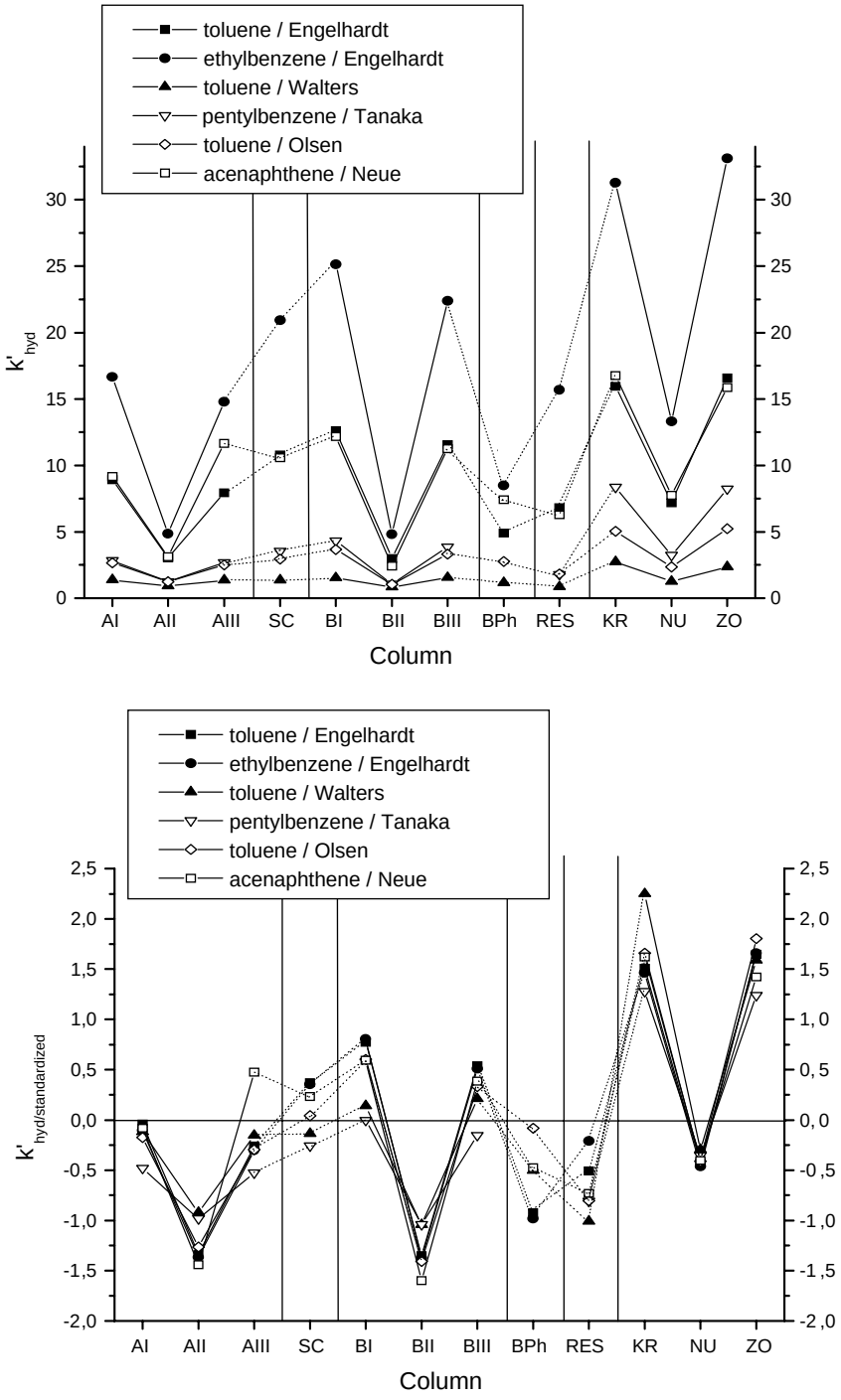

Fig. 2. Comparison of k'_{hyd} and standardized k'_{hyd} values on all investigated columns (k'_{hyd} (pentylbenzene) of BPh was not used, see note in Fig. 3).

classification of the stationary phases (Fig. 2). The lower k'_{hyd} values on AII and BII could be due to the lower carbon loading on these materials (Table 1). Hence, the retention of apolar compounds on these phases was not as strong.

The calix[4]- and calix[8]arene phases have only minor differences in k'_{hyd} values (Fig. 2). This corresponds to the similar carbon contents and surface areas of these materials (Table 1). Interestingly, acenaphthene has a significantly higher retention on AIII than on AI. A similar behavior was not observed on BIII compared to BI. Thus, we suppose that the calix[8]arenes of AIII interact in a special way with this solute, leading to the stronger retardation. With the other test methods a higher hydrophobic retention capacity of AIII compared with AI, BI and BIII was not observed. Hence, the test method of Neue et al. [24] is less appropriate to evaluate the differences between calixarene phases because

special interactions between AIII and acenaphthene can not be excluded.

Regardless of the ring size of the calixarenes, the k'_{hyd} values obtained with the calixarenes of the B-series were higher than those for the A-series (Fig. 2). Because of the high similarity in the carbon loadings and the surface areas we assume that interactions between the alkyl substituents of the solutes and the *p-tert*-butyl groups of the calixarenes take place. These additional interactions can not be formed with calixarenes of the A-series. Furthermore, the cavities of the *p-tert*-butylcalixarenes are deeper and widened compared to the calixarenes lacking these groups. This could lead to a better inclusion of the hydrophobic solutes, which would explain the higher retention on these phases.

As expected, the hydrophobic retention capacities of SC were between AI and BI because this material is a 1:1 mixture of the two (Figs. 1 and 2).

BPh has a similar surface area and carbon content compared to AII and BII (Table 1). Thus, a similar hydrophobic retention capacity should be obtained. But, k'_{hyd} values on this phase are somewhat higher than on calix[6]arene materials (Fig. 2). With acenaphthene as a test solute, the retention capacity is even almost as high as on AI and AIII. In spite of the chemical similarity towards the *p-tert*-butyl calixarenes the selectors on BPh do not have the same rigidness as the calixarenes. Obviously, this is responsible for the differences when methods with different test solutes and chromatographic conditions are used. Hence, the structure of the stationary phase of BPh could be influenced to a greater degree by the mobile phase composition than those of the calixarene phases.

The resorcinarene phase has smaller k'_{hyd} values than the calix[4]- and calix[8]arene phases although the surface area and the carbon loading is very similar (Table 1, Fig. 2). This could be due to the phenolic groups at the upper rim of the cavities leading to a more polar character (Fig. 1). Thus, hydrophobic solutes are less retained than on the calixarene phases.

We observed interesting differences between RES and the calixarene phases when comparing the relative deviations in dependence on the composition of the eluent. With the method of Engelhardt et al. [19] almost the same k'_{hyd} values for RES, AI and AIII are obtained. In contrast, with MeCN containing mobile phases according to Walters et al. [20], a much lower hydrophobic retention capacity is obtained that is as low as with the calix[6]arene phases. Increasing the water content of the methanolic eluents leads to apparent higher hydrophobicities so that the k'_{hyd} values are in the range of AI and AIII, respectively. This confirms the great importance of the chosen chromatographic conditions for an evaluation of this descriptor. Nevertheless, all methods confirm in general that RES has a hydrophobic retention capacity lower than most other phases.

The highest values of k'_{hyd} are obtained on KR and ZO as expected by the high surface areas and carbon contents, respectively (Table 1, Fig. 2). However, NU has a lower hydrophobicity due to the lower surface area and smaller carbon loading. The k'_{hyd} values are in the range of AI and AIII whereas on BI and BIII phases solutes are retained stronger.

The results on the RP-C_{18} phases demonstrate that the chosen methods correspond very well and show no large deviations in dependence on the chromatographic conditions. Thus, an evaluation is simpler than for the calixarene and resorcinarene phases because of the relative independence of the method.

3.2. Hydrophobic selectivity

The hydrophobic selectivity α_{hyd} is a further descriptor characterizing the hydrophobicity of stationary phases. In most cases, the hydrophobic retention capacity and the hydrophobic selectivity should correspond to each other on the investigated phases (Figs. 2 and 3). However, regardless of the retention time this parameter explains better the ability of a stationary phase to distinguish between analytes of similar hydrophobicity. Hence, not every phase with high k'_{hyd} values also have compelling high α_{hyd} values.

Comparing the absolute values, the highest deviations between the stationary phases were obtained with the method of Walters et al. [20]. In contrast, α_{hyd} (pentylbenzene/butylbenzene) obtained according to the method of Tanaka et al. [21] gives just minor absolute differences between the materials. Results were interpreted by the standardized α_{hyd} values analogous to (1) for k'_{hyd} values.

The calix[6]arene phases have the distinct lowest hydrophobic selectivities of all phases due to the low surface areas and carbon loadings (Table 1, Fig. 3). Interestingly, BPh and RES possess higher α_{hyd} values regardless of the chosen method although k'_{hyd} values of these phases are more similar to AII and BII in some test systems. A discussion based on chemical and physical parameters is not conclusive because the values do not differ that much.

All calix[4]arene and calix[8]arene phases have higher hydrophobic selectivities (Fig. 3). Differences dependent on the ring size of the calixarenes were not found. Only with the method of Neue et al. [24] higher values were obtained on AIII than on AI, which corresponds to the discussion of k'_{hyd} values. A reason was given by specific interactions of the calix[8]arenes with acenaphthene. The results with α_{hyd} support this hypothesis. Thus, this test gives no general information concerning hydrophobic properties of AIII but shows the specificity for the selected test solute.

When the phenyl selectivity is used for the evaluation of hydrophobic selectivity the calixarene phases of the A-series have higher values than those of the B-series (Fig. 3). In contrast, higher α_{hyd} values are obtained on *p-tert*-butylcalixarene phases when homologous series are used as test solutes. We assume that interactions between alkyl groups of the analytes and calixarenes are

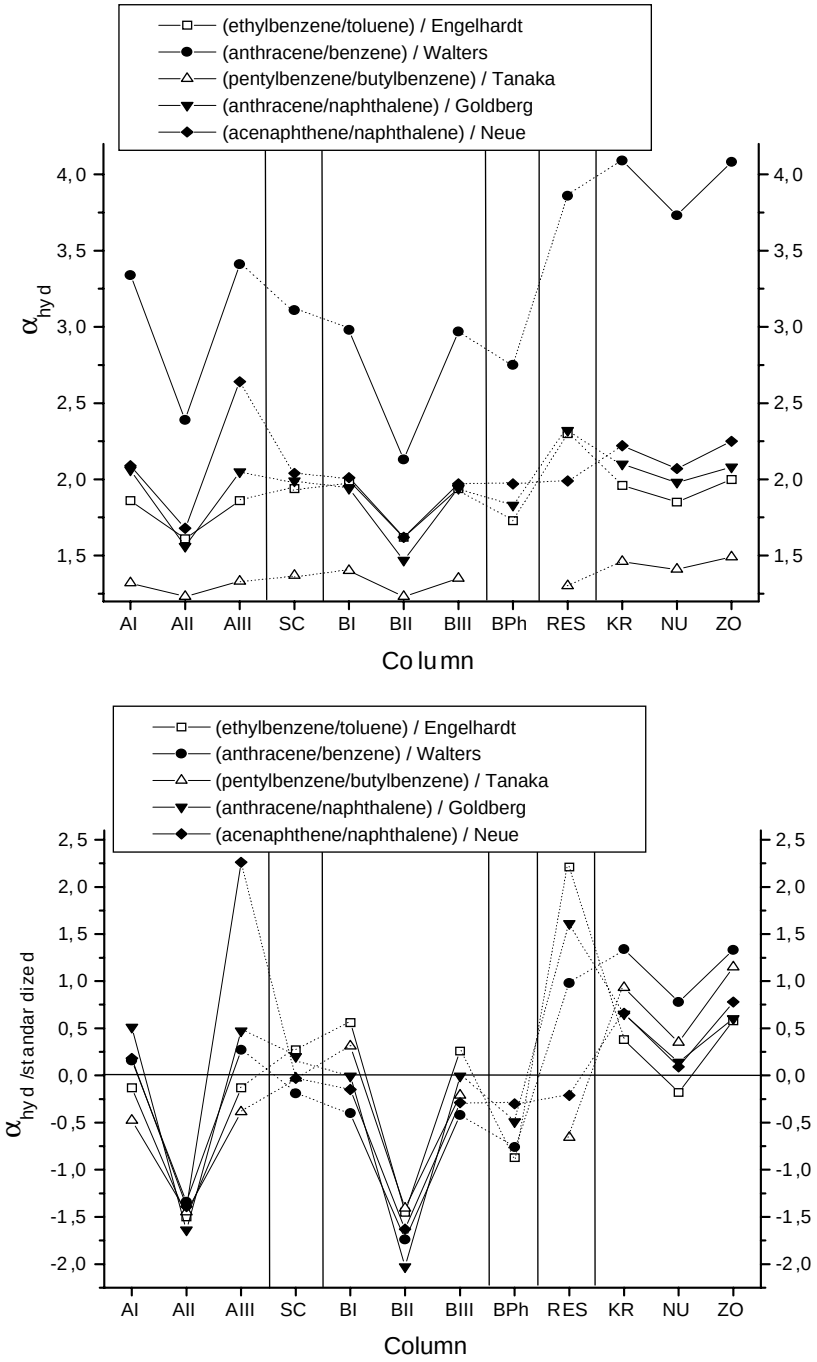

Fig. 3. Comparison of α_{hyd} and standardized α_{hyd} values on all investigated columns (α_{hyd} (pentylbenzene/butylbenzene) of BPh was not used because of strongly differing values compared to the other tests of methylene selectivity).

responsible for the better selectivity on these materials. Obviously, calixarenes without *p-tert*-butyl substituents are better able to discriminate phenylogeous compounds. This could be caused by the absence of *p*-substituents, leading to better interactions between aromatic systems of solutes and calixarenes. An other work tries to explain similar phenomenon by π–π interactions [14]. Thus, a uniform conclusion concerning the differences in hydrophobic selectivity of calixarene phases can not be made here because the tests strongly depend on the type of the test solutes.

BPh has a lower hydrophobic selectivity than the calix[4]arene and calix[8]arene phases (Fig. 3). This corresponds to the lower k'_{hyd} values in most cases (Fig. 2). It is due to the lower carbon content and surface area of the material (Table 1).

In spite of the low hydrophobic retention capacity of RES, highest hydrophobic selectivities of all columns were obtained with some methods (Fig. 3). Otherwise, α_{hyd} (pentylbenzene/butylbenzene) and α_{hyd} (acenaphthene/naphthalene) are in the range of the calix[4]arene and calix[8]arene phases. The higher polarity of RES caused by the phenolic groups at the upper rim should weaken the interaction with hydrophobic solutes. Hence, the high methylene or phenylene selectivity must be due to specific interactions, such as inclusion complexes with the cavities.

As for the hydrophobic retention capacity, KR and ZO possess the highest hydrophobic selectivities of all phases (Fig. 3). NU had lower α_{hyd} values than the other RP-C_{18} phases due to the lower surface area (Table 1). No preference for homologous or phenylogeous groups was observed. In the test according to Walters et al. [20], the phases give the highest values in MeCN containing eluents. However, the α_{hyd} values obtained in the test systems of Goldberg et al. [23] or Engelhardt et al. [19] were not higher than on AI and AIII or BI and BIII, respectively. Eluents with higher contents of MeOH or with MeCN gave better hydrophobic selectivities on these phases.

The differences between the standardized k'_{hyd} values of the RP-C_{18}-phases and the calixarene phases were more distinct than the those differences between the standardized α_{hyd} values. Thus, in spite of greater retention on the octadecyl phases (Fig. 2) the selectivities were not increased accordingly (Fig. 3). This confirms that the calixarene phases have relatively better separation properties for hydrophobic solutes than expected from their hydrophobic retention capacity.

3.3. Steric selectivity

The steric selectivity describes the ability of a chromatographic material to distinguish solutes having different conformations [1]. Often test solutes with planar or aplanar structures [21,25] or with linear and alinear structures [23,26] are used. We chose the first test introduced for this descriptor with diphenyl and *o*-terphenyl as test solutes according to Goldberg et al. [23] (Table 3, Fig. 4). Furthermore, the popular test of Tanaka et al. [21] with triphenylene and *o*-terphenyl was investigated. The well-known test of Sander and Wise [25] with benzo[a]pyrene, 1,2:3,4:5,6:7,8-tetrabenzonaphthalene and the aplanar phenanthro[3,4-c]phenanthrene was not used because corresponding answers for the steric selectivity of

Table 3
Chromatographic conditions of chosen test methods for the determination of steric selectivities α_{ster}

Author	Steric selectivity α_{ster}	Mobile phase (v/v)
Tanaka [21]	α (triphenylene/*o*-terphenyl)	MeOH/H_2O, 80:20
Goldberg [23]	α (diphenyl/*o*-terphenyl)	MeOH/H_2O, 90:10

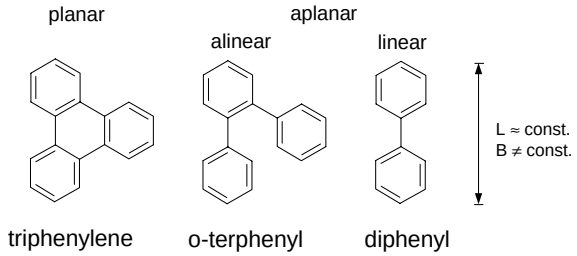

Fig. 4. Chemical structures of test solutes for the determination of the steric selectivity α_{ster} (L: length, B: breadth).

RP-C_{18} materials compared to the test of Tanaka et al. [21] were observed by Engelhardt et al. [27].

The selectivities α_{ster} were referred to *o*-terphenyl. Thus, high α_{ster} values express a high interaction potential of the phases towards planar and linear molecules compared to the aplanar and alinear *o*-terphenyl.

Although α_{ster} values obtained by the methods of Tanaka et al. [21] and Goldberg et al. [23] should not correspond very well [4], we found a good correlation for most materials between the two descriptors (Fig. 5). Only the three calixarene phases of the A-series and BII deviated from the correlation line. The discussion of differences by means of the "slot model" of Sander and Wise [26] is problematic. This model was developed for RP-C_{18}-phases to explain the higher retention of planar molecules on phases with a high surface coverage. A formation of "slots" between calixarene molecules in a similar way is unlikely because calixarenes are more rigid than octadecyl chains with a lower degree of order. More likely, calixarenes and resorcinarenes interact more specific with single analytes than RP-C_{18}-phases because of their own intramolecular cavities. Hence, the application of this descriptor to calixarene and resorcinarene phases for a determination of "steric selectivity" is somewhat problematic.

The α_{ster} values of Caltrex A-phases are greater than those of Caltrex B-phases when the method of Tanaka et al. [21] is used (Fig. 5). Hence, an interaction of calixarenes without *p-tert*-butyl groups at the upper rim with the rigid triphenylene is preferred in comparison to *o*-terphenyl, which has more flexibility. We suppose that calixarenes of the A-series interact better by π–π interactions. This hypothesis is confirmed by the results in Section 3.2, showing a better phenyl selectivity of these materials compared to *p-tert*-butylcalixarene phases.

In contrast, there are hardly any differences between the two series in their ability to discriminate diphenyl and *o*-terphenyl (Fig. 5). Thus, no series has better properties concerning the discrimination of linear and alinear solutes.

The calix[6]arene phases have the lowest steric selectivity according to the test of Tanaka et al. [21]. The high values of α_{ster} obtained with the method of Goldberg et al. [23] correspond to a poor separation of these solutes on the two phases. We assume that this behavior is due to the small

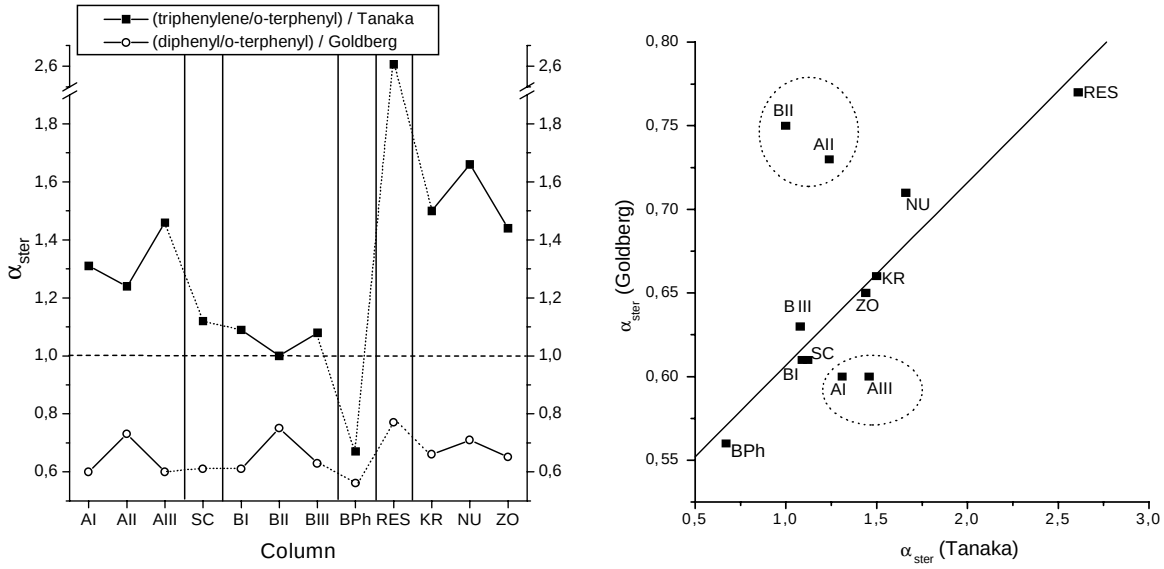

Fig. 5. Comparison of α_{ster} values on all investigated columns.

retention of the analytes because of the low surface coverage (Table 1).

The distinct highest steric selectivities with triphenylene and *o*-terphenyl were obtained on RES (Fig. 5). In addition, linear molecules are stronger retained on this phase compared to alinear ones. This is also an indication for a better steric selectivity, although the resolution of diphenyl and *o*-terphenyl is lower than on the other phases. However, the behavior can not be explained by the "slot model" [26] because the retention mechanism is likely to be influenced by inclusion into the cavities of resorcinarenes. Nevertheless, the test shows the enormous selectivities that can be obtained on this phase if single solutes likely form a special complex with these supramolecules.

BPh has the lowest steric selectivity of all investigated phases (Fig. 5). The low affinity for planar molecules is most distinctly demonstrated in a reversal of the retention order between *o*-terphenyl and triphenylene. Furthermore, the retention of linear molecules is smaller than those of alinear solutes. As discussed for calix[6]arene phases, the low surface coverage could be one reason for this behavior. Additionally, the high flexibility of the bonded ligands compared to the calixarenes and resorcinarenes should to be considered, allowing no host–guest complexations. These two factors could be responsible for the differences between BPh and the phases with supramolecular selectors.

The steric selectivity of monomeric RP-C_{18}-phases is hardly dependent on the methylene selectivity [21]. This could explain why the differences between NU, ZO and KR are not as high as observed for the hydrophobic retention capacity or hydrophobic selectivity (Fig. 5). NU has even the highest steric selectivity of all phases although the hydrophobicity is lower than on the other two phases.

The steric selectivity of the investigated RP-C_{18}-phases is lower than on the resorcinarene phase. However, higher α_{ster} values of these kind of phases are in principle obtainable with materials with higher surface coverage [27–29]. Such materials were not chosen because the characteristics compared to the calixarene and resorcinarene phases (Table 1) had been too different.

Some opportunities are discussed to enhance the steric selectivity of RP-C_{18}-materials. Such as, decreased temperatures [30–32] and addition of *n*-hexanol [30] can lead to higher shape selectivities by a higher degree in the order of the octadecylchains. Investigations of these factors could also increase α_{ster} values of calixarene or resorcinarene phases. Though, it is possible that additives like *n*-hexanol could cause a blockade of the calixarene cavities by absorption into the stationary phase which would diminish steric selectivity. Work is in progress to study these influences.

A direct comparison of the results on RP-C_{18}-materials with those on calixarene and resorcinarene phases is problematic because the interpretation needs the same base retention model for this descriptor. Thus, the usefulness of this parameter to evaluate the properties of the new phases is limited.

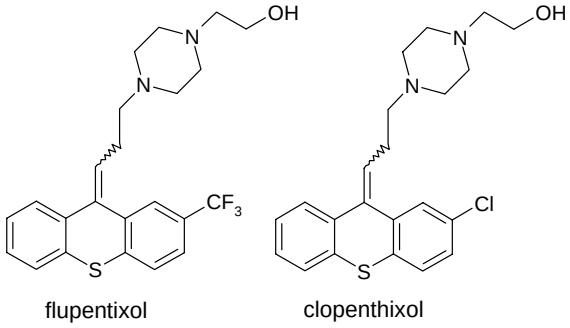

Fig. 6. Chemical structures of thioxanthenes.

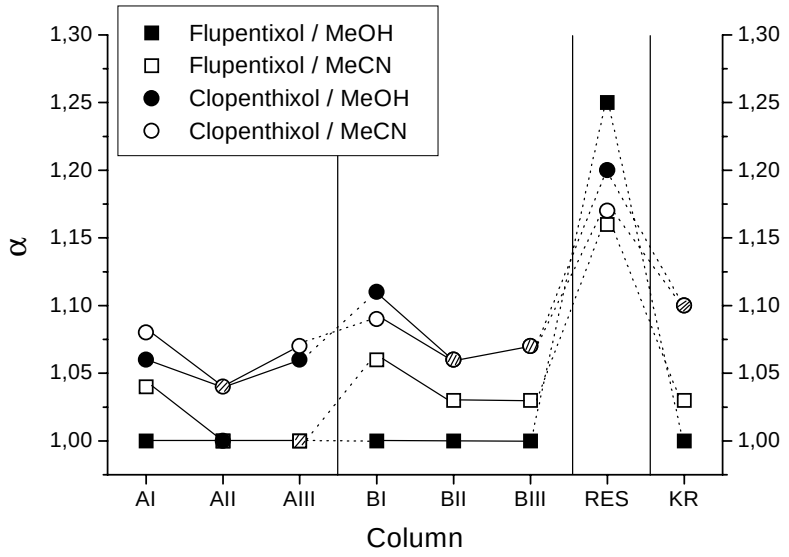

Fig. 7. Separation of flupentixol and clopenthixol, Conditions: MeOH/20 mM NaH_2PO_4 (pH = 3.5) (v/v): calixarene phases: 55:45 (flupentixol), 50:50 (clopenthixol); RES: 55:45; KR: 60:40, MeCN/20 mM NaH_2PO_4 (pH = 3.5) (v/v): calixarene phases: 30:70; RES, KR: 35:65, 225 nm, 1 ml/min, 40 °C.

3.4. Separation of thioxanthenes and steroids

A previous study on thioxanthenes with hydroxyethyl substituents [13] was used to confirm the results with the test mixtures. (*Z*)- and (*E*)-isomers of flupentixol and clopenthixol (Fig. 6) possess about the same lipophilicity. Hence, special demands on the selectivity of the chromatographic materials are necessary to separate these isomers with different neuroleptic activity [33–35].

Six calixarene phases (AI–AIII, BI–BIII) and the resorcinarene phase were compared with KR, which had the best hydrophobic retention capacity and hydrophobic selectivity of all phases. Furthermore, this material is made of the same base silica. Thus, a discussion of the influence of silanol activity (acidity of silanol functions, metal content) is not necessary. The content of organic modifier of the eluent was slightly varied on different materials. Thus, almost the same retention times were obtained on all phases.

The resorcinarene phase was most appropriate to separate the isomers under given conditions (Fig. 7). For example, flupentixol isomers were only separated with MeOH containing eluents on RES whereas all other phases did not have any selectivity with the same organic modifier. In contrast, a discrimination of the isomers of clopenthixol was achieved on all investigated phases. Again, highest separation factors were obtained on RES. The selectivity of KR was a little bit better compared to the calixarene phases.

This order in the selectivities corresponds to the results obtained for the steric selectivity in Section 3.3. Hence, the results of the study can give certain orientation about column choice for a given problem when isomers with differences in the shape have to be separated. On the other hand, other examples demonstrate that other factors can lead to different selectivities. For example, the superior selectivity of calixarene phases compared to KR for

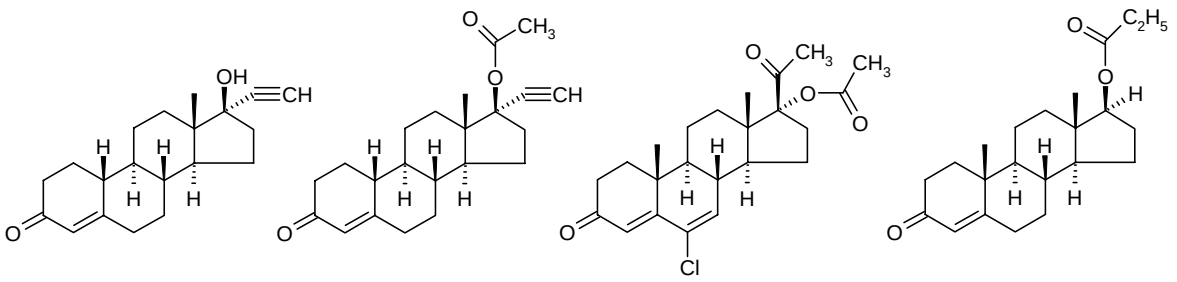

Fig. 8. Chemical structures of gestagenes and testosterone propionate (*c* log *P* values from [28]).

Table 4
Capacity factors (k') and separation factors (α) of the steroid mixture obtained on various columns

	80%[a]												65%[a]		80%[a]	
	AI		AII		AIII		BI		BII		BIII		RES		KR	
	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α	k'	α
Norethisterone	0.56		0.24		1.12		0.61		0.48		0.56		0.85		0.34	
Chlormadinonacetate	1.16	2.07	0.45	1.90	2.89	2.59	1.24	2.01	0.85	1.78	1.09	1.95	1.98	2.33	0.94	2.78
Norethisteronacetate	1.10	0.95	0.45	1.00	2.56	0.89	1.41	1.15	1.00	1.17	1.25	1.15	1.86	0.94	0.94	1.00
Testosteronpropionate	2.12	1.92	0.86	1.94	5.25	2.05	2.93	2.07	1.94	1.94	2.58	2.07	3.95	2.12	3.18	3.38

Conditions: MeOH/20 mM NaH_2PO_4 buffer (pH = 3.5) in different proportions, 260 nm, 1 ml/min, 40 °C.
[a] MeOH content.

the discrimination of benzo[b,e]oxepin isomers and other thioxanthene isomers with a different substitution pattern is described [13]. Reasons were given by polar interactions with the stationary phases, differences in the flexibility of the calixarenes and the kind of the tricyclic ring system of the isomers with different lipophilicity and π-electron density.

Another example of the differentness of calixarene and resorcinarene phases is given by a separation of a mixture of steroids (Fig. 8). All steroids were without basic or acidic functions. Thus, the main retention mechanism should be based on hydrophobic interactions with the chromatographic materials. The retention of solutes is stronger on phases with higher hydrophobicity resulting in better resolutions of neighboring pairs of analytes.

In contrast, polar functions of the steroids, such as carbonyl or alcohol groups, should play a smaller role in the retention mechanism. Nevertheless, interactions of these groups with polar parts of the stationary phases, such as silanol functions or the phenolic groups of the resorcinarenes, must be considered.

The mixture contained three steroids with gestagenic properties [36] (Fig. 8). Because of the chemical similarity, testosterone propionate was chosen as androgen. It was found that all steroids could be separated on all calixarene and resorcinarene phases with exception of AII (Table 4, Fig. 9). There was a change of the elution order of the two analytes on calixarene phases of series A and B. The retention on RES corresponds to Caltrex A-phases.

In contrast, no separation of chlormadinone acetate and norethisterone acetate was obtained on KR (Table 4, Fig. 9). Chlormadinone acetate and norethisterone acetate have almost identical $c\log P$ values [37] (Fig. 8). Thus, a separa-

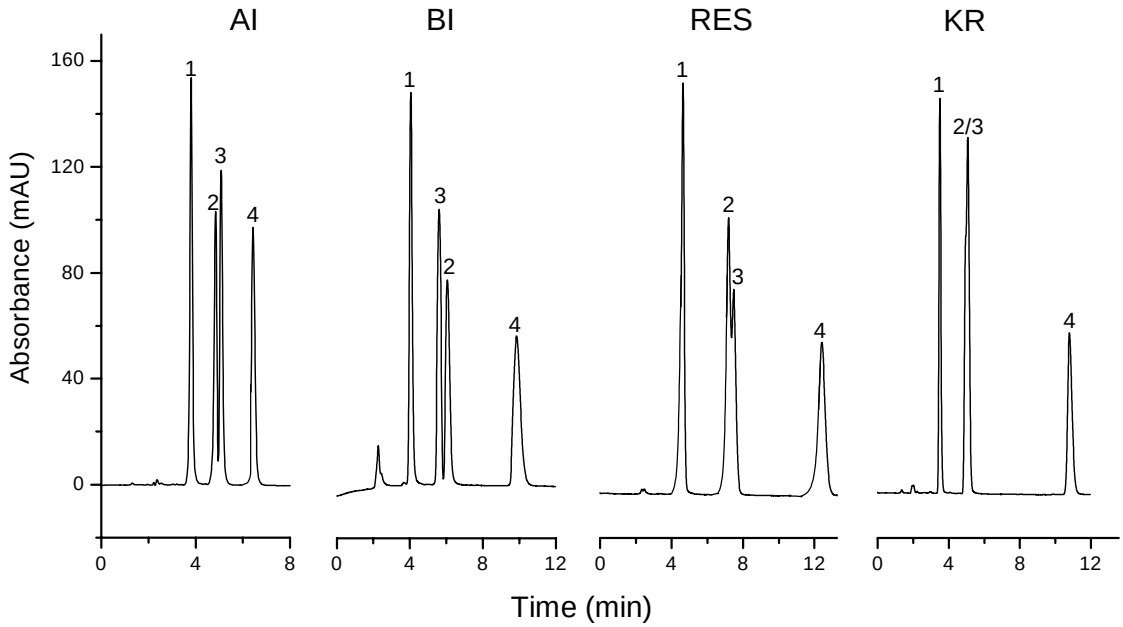

Fig. 9. Separation of steroids on AI, BI, RES and KR, analytes: (1) norethisterone, (2) norethisterone acetate, (3) chlormadinone acetate, (4) testosterone propionate. Conditions: AI, BI, KR: MeOH/20 mM NaH_2PO_4 (pH = 3.5) 80:20 (v/v), RES: MeOH/20 mM NaH_2PO_4 (pH = 3.5) 65:35 (v/v), 260 nm, 1 ml/min, 40 °C.

tion of these analytes on hydrophobic phases needs a high selectivity of the chromatographic materials.

It was found that the high hydrophobic selectivity of KR is not a good criterion to predict a good separation of the two steroids with similar lipophilicity on this phase. Otherwise, in spite of the lower hydrophobic selectivities of the calixarene and resorcinarene phases better resolutions of this pair of analytes were obtained. The elution order on Caltrex B-phases corresponds to the order of $c\log P$ values of the steroids. In contrast, on Caltrex A-phases chlormadinone acetate has a higher retention in spite of the lower hydrophobicity than norethisterone acetate. We assume that specific interactions with the cavities of the calixarenes and resorcinarenes are responsible for these phenomenon. HR–MAS–NMR studies confirm a stronger interaction of chlormadinone acetate with AIII in comparison to BIII, which was due to better π–π interactions [38]. Thus, phases with supramolecular selectors are able to detect differences of the analytes with similar lipophilicity. This behavior is disregarded in the hydrophobic parameters obtained by empirically based test mixtures. Hence, an evaluation of calixarene and resorcinarene phases by these descriptors stays difficult for a prediction of the selectivity of unknown mixtures because specific interactions can cause a much better resolution than expected by k'_{hyd}, α_{hyd} or α_{ster}.

4. Conclusions

The evaluation of hydrophobic properties of calixarene and resorcinarene phases by means of empirically based test mixtures was performed for the first time. The calixarene phases have a lower hydrophobic retention capacity and a somewhat lower hydrophobic and steric selectivity than RP-C_{18}-phases with higher surface coverages. Nevertheless, the supramolecular phases showed distinct differences when different test mixtures and test conditions were used; i.e. there is a higher phenyl selectivity and a lower methylene selectivity on Caltrex A-phases compared to Caltrex B-phases. To some degree the phases showed a much higher selectivity for some test solutes, such as observed in the determination of α_{hyd} and α_{ster} on RES. This behavior was due to specific interactions of the supramolecular receptors with some analytes.

The separation of geometric isomers of two thioxanthenes corresponded to results obtained from the studies with test mixtures. Against it, the separation of a mixture of steroids demonstrated a much higher selectivity of calixarene and resorcinarene phases compared to the Kromasil-C_{18}-phase to discriminate molecules of similar lipophilicity. Thus, an evaluation of the new phases by means of empiric test mixtures does not take into consideration specific interactions occurring between supramolecular cavities and analytes. Hence, test runs with the known methods can give some orientation for the choice of a chromatographic material. But as only parameters, they are insufficient to evaluate the real potential for separation.

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