NEW CALIX[4]ARENE SILICA GEL BONDED STATIONARY PHASES. CHARACTERIZATION AND APPLICATION TO HPLC.

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INTRODUCTION

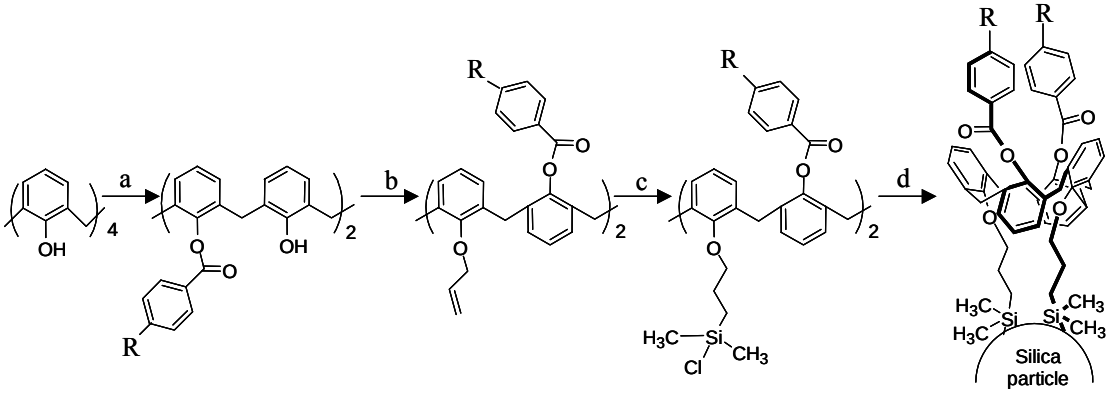
The ability to form complexes with other molecules of suitable size and hydrophobicity makes the calixarenes a useful class of stationary phases. The potential of these macrocyclic compounds for applications in gas chromatography [1-2], capillary electrophoresis [3-4], solid phase extraction [5] and high-performance liquid chromatography [6-10] has been shown in last years. Calixarene-bonded stationary phases in a cone conformation are excellent in reversed-phase chromatography. Recently, we have reported synthesis of three calix[4]arene-bonded silica gel stationary phases in *1,3-alternate* conformation (*1,3-Alt* CalixPr, *1,3-Alt* CalixBn, *1,3-Alt* CalixBz) and we have confirmed that these phases posses high selectivity toward selected analytes [13-14]. In this paper we described the synthesis of two novel *1,3-alternate* 25,27-bis-[*p-tert*butylobenzoiloxy]-26,28-bis-[3-propyloxy]-calix[4]arene (Calix *t*Bu) and *1,3-alternate* 25,27-bis-[*p*-methoxybenzoiloxy]-26,28-bis-[3-propyloxy]-calix[4]arene (Calix OMe) bonded silica gel stationary phases and their application to resolution of aromatic positional isomers, purine and pyrimidine bases as well as water soluble vitamins. The efficiency and selectivity comparison of these phases has been investigated.

EXPERIMENTAL SECTION

The synthesis of new calix[4]arene stationary phases is shown in Scheme 1. Elemental analyses of modified silica gels gave 19.86 %C, 2.04 %H (coverage density of the gel 0.344 mmol g^{-1}) for Calix *t*Bu and 18.33%C, 2.31 %H (coverage density of the gel 0.352 mmol g^{-1}) for Calix OMe. Stainless steel columns (150 x 4.6 mm I.D.) were packed with modified calix[4]arene-silica gels according to a slurry packing procedure . The columns efficiency of about 30 000 plates/meter was determined with a commercially available test mixture.

Chromatographic procedure:

Liquid chromatograph HP Series 1090 (Hewlett-Packard Inc.) equipped with quaternary pump, autosampler, thermostated column compartment and diode-array detector was used. Analytes were dissolved in MeOH/H_2O (1:1 v/v) mixture at the concentration in range of 0.25 to 0.5 mg ml^{-1} and 5 µl of the solution were injected onto the chromatographic column. A mixture of MeOH in water or in 0.01 mol l^{-1} water solution of phosphate buffer was used as a mobile phase at flow rate of 1.0 ml min^{-1}. Diode array detector was operated in single wavelength mode. All analyses were carried out at 30º C.



Scheme 1. Synthesis of the *1,3-alternate* Calix *t*Bu and Calix OMe silica gel stationary phases; (a) p-substituted benzoyl chloride, ACN, K_2CO_3, reflux for 24 h; (b) allyl iodide, Cs_2CO_3, ACN, reflux for 72 h; (c) $(CH_3)_2SiHCl$, H_2PtCl_6, $CHCl_3$, reflux for 4 h; (d) activated silica gel, pyridine, shaking for 4 days at room temperature. Substituents R: *tert*-butyl or methoxyl.

RESULTS AND DISSCISSION

Both novel stationary phases exhibit strong retention power and selectivity toward analytes of very similar structure. A variety of aromatic positional isomers, representing compound with acidic, basic and neutral character, were well resolved. Retention capacity factors (k) and separation factors (α) of these isomers at the best, individually optimized, chromatographic conditions are given in Table 1.

Table 1. Retention and separation factors for benzene positional isomers on Calix *t*Bu and Calix OMe stationary phases.

		Calix *t*Bu		Calix OMe	
Analytes	**Isomers**	**k**	**α**	**k**	**α**
Hydroxypyridine [A]	*para*	0,05	9,98 2,71	0,09	4,60 1,11
	ortho	0,50		0,42	
	meta	1,35		0,88	
Aminobenzhydrazide [A]	*para*	0,32	2,78 1,35	1,06	1,07 1,69
	meta	0,90		1,14	
	ortho	1,20		1,92	
Hydroxybenzyl alcohol [B]	*para*	1,43	1,43 1,45	1,47	1,23 1,22
	meta	2,04		1,80	
	ortho	2,96		2,20	
Aminophenol [B]	*para*	0,74	1,44 1,82	0,99	1,10 1,41
	meta	1,07		1,08	
	ortho	1,94		1,61	
Nitroaniline [C]	*para*	2,99	1,13 1,48	2,65	1,02 1,48
	meta	3,39		2,69	
	ortho	5,01		4,01	
Hydroxybenzoic acid [D]	*ortho*	4,08	1,09 1,51	1,18	1,06 2,07
	meta	4,45		1,26	
	para	6,72		2,60	
Chlorobenzoic acid [D]	*ortho*	0,25	9,41 1,08	1,03	3,19 1,06
	meta	2,34		3,28	
	para	2,54		3,47	
Nitrobenzoic acid [E]	*ortho*	1,22	5,71 1,05	1,81	5,75 1,17
	meta	6,95		10,43	
	para	7,33		12,22	

Mobile phases: A- H_2O/MeOH (9:1 v/v); B- H_2O/MeOH (8:2 v/v); C- H_2O/MeOH (6:4 v/v); D- 10mM phosphate buffer at pH 3.5/MeOH (9:1 v/v); E-10mM phosphate buffer at pH 3.5/MeOH (3:7 v/v)

As can be seen, compounds possessing nitro substituents in the phenyl ring have higher retention times in comparison to the rest of investigated analytes. This may be explained by π-electron transfer interaction resulting from the electron-withdrawing effect of the nitro groups of analytes and π-electron system of the *para*-substituted benzoiloxy groups of calixarene. The elution order of positional isomers of the investigated compounds on calixarene columns strongly depends on their chemical nature. Polar neutral, basic and weak acidic compounds (for example dinitrobenzene, nitroaniline and nitrophenol) were eluted on both columns in order: *para* < *meta* < *ortho*, which may be associated to guest-host interactions of the calixarene cavity and the analyte molecule. Only *ortho* -hydroxypyridine was eluted before *meta* isomer (*para*>*ortho*>*meta*). It can be explained by ability of *ortho* and *para* isomers of hydroxypyridine to exist in tautomeric form of pyridin-(1H)-one. The elution order of the substituted benzoic acid isomers was opposite (*ortho*>*meta*>*para*) and follows pK_a values order of these acids. Capacity factor values of the most separated isomers are greater on Calix OMe column than on Calix *t*Bu phase. It may be due to stronger electro donating properties of methoxyl substituents present in aromatic ring of calixarene molecules at *para* position. Moreover, hydrogen bond interaction between the methoxyl group and hydrogen-donor (OH, NH_2) isomers can play additional role in the discrimination process.

The chromatographic performance of Calix OMe and Calix *t*Bu stationary phases was also evaluated using nucleo bases and water-soluble vitamins separation. Figures 2 and 3 show typical chromatograms of above-mentioned analytes.

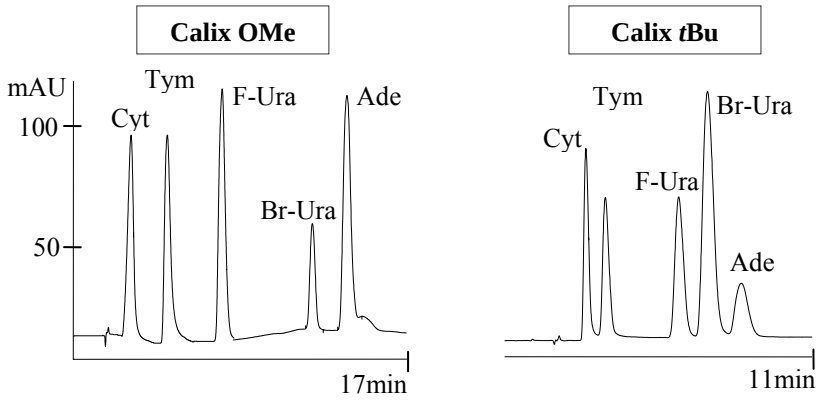


Figure 2. Separation of purine and pyrimidine bases. Mobile phase: methanol-10mM phosphate buffer at pH= 6 (1:9 v/v), flow 1ml min^{-1}, UV 254 nm, T=25^0C.

Mixture of five bases was separated completely on both calixarene columns (Fig.2). Elution order of the analytes was the same however, solutes are a little more retained on Calix OMe and peaks are sharper.

The separation of seven water-soluble vitamins (Fig.3) can be achieved in isocratic mode within 16 minutes. It must be noted that such results is very difficult to achieve on ODS column, where retention mechanism based only on hydrophobicity of the analytes.

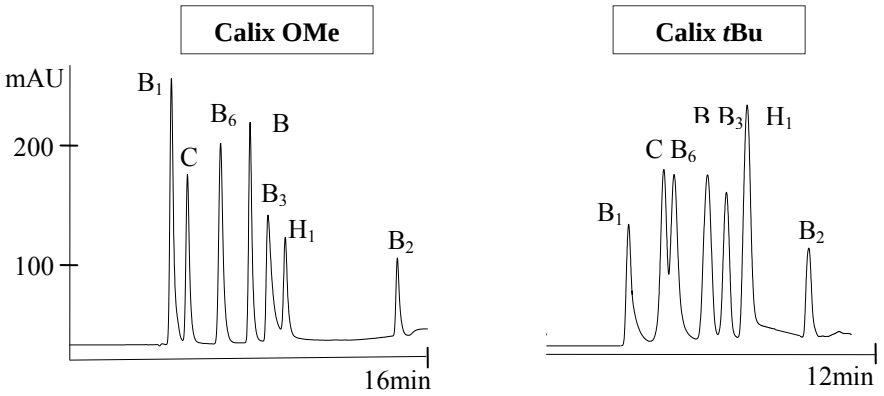


Figure 3. Separation of water-soluble vitamins. Mobile phase: with methanol-phosphate buffer at pH=3.5 (10:90 v/v), flow 1ml min^{-1}, UV 254 nm, T=25^0C.

Better separation of vitamins was observed on Calix OMe, which exhibited stronger retention power and better resolution toward the analytes. Separation of vitamins C and B_6 were not complete on Calix *t*Bu.

CONCLUSION

Screening evaluation of *1,3*-alternate Calix *t*Bu and Calix OMe revealed that the new stationary phases are chemically stable and can be successfully used for separation of positional isomers of aromatic compounds. The applicability of the novel chromatographic columns to analysis of selected biologically vital compounds was also demonstrated. Investigation of surface properties of calixarene stationary phase and retention mechanism is in progress.

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