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Activated silica supports for preparation of chromatographic sorbents. A comparative study of silicas containing attached epoxy, tosyloxy and halogen groups

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Abstract

A comparative study of silica sorbents with attached epoxy, tosyloxy and halogen groups is presented. Epoxy-activated sorbents are shown to have the lowest concentration of attached functional groups among the activated sorbents studied. The low content of epoxy groups is suggested to originate from the partial destruction of epoxy rings during the modification of silica. New methods of epoxidation without the use of epoxysilanes and tosylation without the use of tosyl chloride are proposed. Haloalkyl-activated sorbents are shown to have the highest content of attached functional groups in a bonded layer. It is suggested that haloalkyl-activated sorbents are the most promising for the preparation of sorbents with high loadings of immobilized ligands.

1. Introduction

Chemically modified silicas are widely used as stationary phases for normal-phase, reversed-phase, ion-exchange, ligand-exchange, affinity and other chromatographic methods [1–5] and syntheses of a variety of functionalized silica sorbents have been described [1–3,6]. Silica stationary phases are usually prepared using the “surface chemical assembly” technique, which involves reactions between the immobilized ligand on the one hand and the silica modified with a simple functionalized organosilicon compound on the other [1,3,5,6]. Such silicas are referred to as activated silicas.

Activated silicas containing epoxy or halogen

functional groups are of most interest for the preparation of stationary phases, for the following reasons. First, the majority of immobilizing ligands have groups such as NH_2 , HS and HO , which react readily with epoxides and haloalkanes [1,3–12]. Second, the resulting C–N–C, C–S–C or C–O–C bonds are hydrolytically stable [1,6,7,13]. Third, these activated silicas can be easily obtained via the reaction between silica and readily available organosilicon compounds [1,3,6].

This work was devoted to the study of both the advantages and drawbacks of epoxy- and haloalkyl-activated silicas. In addition, we studied tosyl-activated silica, which is of great interest as tosylates react readily with the majority of amine- and thiol-containing ligands. Further, tosylated organic matrices have been suc-

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cessfully used in preparation of stationary phases for affinity chromatography [5].

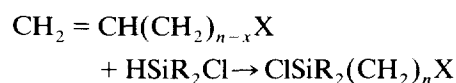
2. Experimental

2.1. Silica

The silica support Silokhrom S-120 (Luminophor, Stavropol, Russian Federation) has an average pore diameter of 35–40 nm, a BET specific surface area of 116 m²/g and a pore volume of 1.05 ml/g. The structural characteristics were obtained from the benzene adsorption isotherm at room temperature as described [14]. The fraction of 0.35–0.50 mm was used.

2.2. Synthesis of silanes

Bromomethylmethyldichlorosilane was obtained by photochemical bromination of dimethyldichlorosilane [15]. Haloalkyltrichlorosilanes and acetoxyundecyltrichlorosilane were obtained by catalytic hydrosilylation of the corresponding alkenes with trichlorosilane [16]:



R = Cl, CH₃; X = Hal, n = 3,5; X = OAc, n = 11

3-Bromopropyldimethylchlorosilane was obtained according to the same equation but using dimethylchlorosilane instead of trichlorosilane [16].

3-Glycidooxypropyltriethoxysilane (GOPTES), vinyltrichlorosilane (both produced by Reakhim, Moscow, Russian Federation) and bromomethylmethyldimethylchlorosilane (Merck, Darmstadt, Germany) were additionally purified by distillation.

2.3. Chemical modification

Bromomethylmethyldichlorosilane, bromomethylmethyldimethylchlorosilane, 3-bromopropyltrichlorosilane, 3-bromopropyldimethylchlorosilane, 5-bromoamyltrichlorosilane and 11-chloroundecyltrichlorosilane.

A 1-g amount of vacuum-dried silica was stirred in boiling anhydrous toluene containing 1

mmol of modifier for 10 h. The sorbents were then extracted consecutively with anhydrous toluene, toluene, acetone, acetone–water (1:1) and acetone and subsequently extracted in a Soxhlet apparatus with diethyl ether for 6 days.

2.3.1. Vinyltrichlorosilane

A 1-g amount of vacuum-dried silica was stirred in boiling anhydrous toluene containing 1 mmol of modifier and 5 mmol of anhydrous morpholine for 10 h. The sorbents were then extracted consecutively with anhydrous toluene, toluene, acetone, acetone–water (1:1) and acetone and subsequently extracted in a Soxhlet apparatus with diethyl ether for 6 days.

2.3.2. 3-Glycidyoxypropyltriethoxysilane

A 1-g amount of silica was stirred in 100 ml of 0.1 M acetate buffer (pH 5.5) containing 1 mmol of modifier for 2 h at 95–100°C. The sorbent was then extracted consecutively with distilled water and acetone.

Syntheses under anhydrous conditions were carried out as for chlorosilanes.

2.3.3. Epoxidation of vinylsilica

A 1-g amount of vinylsilica was added to 10 ml of epoxidizing mixture (as indicated below) and the mixture was allowed to stand at room temperature in the dark for 2–48 h. The sorbents were then extracted consecutively with acetic acid, distilled water and acetone.

The following epoxidizing mixtures were tested in the epoxidation reactions of vinylsilicas. (i) A mixture of glacial acetic acid and 13% H₂O₂ [3:1 (mol/mol, relative to 100% H₂O₂)] was allowed to stand at room temperature in the dark for 2 days. (ii) Glacial acetic acid was added with stirring to a mixture of acetic anhydride and 13% H₂O₂ [2:1 (mol/mol, relative to 100% H₂O₂)] until the mixture was homogeneous. Addition of glacial acetic acid was needed because 13% H₂O₂ and acetic anhydride are immiscible liquids. The mixture was allowed to stand at room temperature in the dark for 5 days. (iii) Glacial acetic acid was added with stirring to a mixture of acetic anhydride and 25% H₂O₂ [1:1 (mol/mol, relative to 100% H₂O₂)] until the mixture was homogeneous. Addition of

glacial acetic acid was needed because 25% H_2O_2 and acetic anhydride are immiscible liquids. The mixture was allowed to stand at room temperature in the dark for 5 days.

2.3.4. Attached alcohols

(i) A 1-g amount of silica modified with 11-acetoxyundecyltrichlorosilane was added to 100 ml of 50% sulphuric acid and allowed to stand for 2–3 h. It should be noted that 50% sulphuric acid does not wet the sorbent, which contains acetoxyundecyl attached groups. However, 50% sulphuric acid does wet the sorbent, which contains hydroxyundecyl attached groups. After 2–3 h the sorbent had fallen to the bottom of the reaction flask, indicating that the reaction was complete. The sorbent was then immediately removed from the acid and extracted with distilled water until a neutral reaction of the supernatant was observed.

(ii) A 1-g amount of silica modified with GOPTES was stirred with 100 ml of water containing sulphuric acid (pH 2.0) for 2 h. The sorbent was then extracted with distilled water until a neutral reaction of the supernatant was observed.

2.3.5. Tosylation of attached alcohols

A 1-g amount of silica with attached alcohol groups [for synthesis see *Attached alcohols*, (i) and (ii)] was extracted with dry acetone on a glass Schott filter. The sorbent was then added to a mixture containing 5 ml of dry acetone, 0.5 g of tosyl chloride and 0.3 g of dry pyridine. The mixture was allowed to stand in an ice-bath for 24 h, then the sorbent was extracted with acetone (6–8 times \times 10–15 ml) until the disappearance of the odour of tosyl chloride.

2.3.6. Tosylation of epoxy-activated silica with *p*-toluenesulphonic acid

A 1-g amount of silica modified with GOPTES was stirred with 50 ml of DMFA containing 0.5 g of *p*-toluenesulphonic acid at 90°C for 5 h. The sorbent was then extracted with distilled water and acetone.

2.4. Determination of concentration of surface functional groups

2.4.1. Combustion analysis

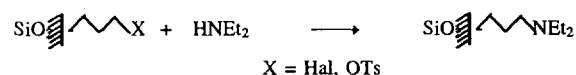
C,H analysis was carried out for all types of sorbents studied. The concentration of attached groups was determined as follows [1–3]:

$$c(\text{groups}/\text{nm}^2) = 6 \cdot 10^5 P_C / [(1200n_C - WP_C)S]$$

where c is the concentration of attached groups, which contain carbon; P_C is the percentage of carbon in the sample, n_C is the number of carbon atoms in the attached group, W is the corrected formula mass of the modifier [1] and S is the specific surface area (m^2/g).

2.4.2. Titration with diethylamine

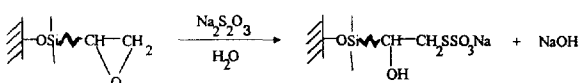
A 1-g amount of a sorbent was added to 10 ml of anhydrous diethylamine and allowed to stand at room temperature for 2 days. The reaction between diethylamine and attached epoxides, haloalkyls and tosylates results in the formation of attached tertiary amines [1,6,17,18]:



The sorbent was extracted consecutively with 10% HCl, saturated NaHCO_3 solution, distilled water, 2-propanol and pentane, then dried at 50°C for 2 h. The attached tertiary amines was titrated with 0.01 M HCl in 1 M NaCl up to a pH of the mixture of 3.7. The amount of attached amine (and therefore the amount of corresponding initial tosylate, epoxy or haloalkyl groups) is equal to the amount of HCl needed for titration [1,19].

2.4.3. Titration with sodium thiosulphate

This method was used only for epoxy-activated silicas. A 1-g amount of the sorbent was added to a saturated aqueous solution of sodium thiosulphate. Attached epoxides reacted as follows [20]:

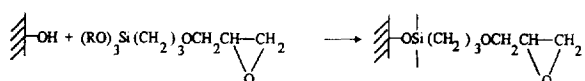


The resulting NaOH was titrated with 0.01 M HCl. The amount of HCl needed for titration is equal to the amount of attached epoxides [20].

3. Results and discussion

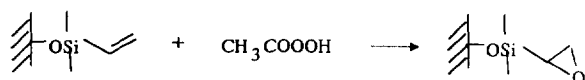
3.1. Epoxysilicas

3-Glycidooxypropyltrialkoxysilanes (GOP-TAS) are commonly used reagents for the preparation of epoxy silicas [1,6,21,22]:



This reaction was studied in detail by Porsch [22]. It was shown that glycidyoxypropyl groups may participate in oxirane ring opening and form a variety of products [22]. Our results agree well with those obtained by Porsch [22]. To obtain epoxy-activated silica with a high loading of oxiranes, one can use the procedure described by Porsch [22] (aqueous solution of GOPTAS, pH 8.5).

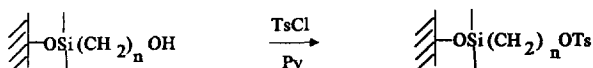
In this study, we tested another technique for the synthesis of an epoxy silica. It involves the epoxidation of attached vinyl groups with peracetic acid, which is formed in situ:



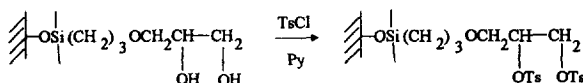
The results obtained are given in Table 1, and show that concentrations of epoxides up to 2.2 groups/nm² can be obtained with a mixture of acetic anhydride and 25% H₂O₂ in a molar ratio of 1:1 (relative to 100% H₂O₂).

3.2. Tosylsilica

These sorbents were obtained by the tosylation of attached alcohols [23]:



We found that both hydroxyl groups were substantially substituted to tosyloxy groups if a diolsilica was taken:



A double tosylation mechanism follows from the titrimetric data given in Table 2. Indeed, assuming one-site tosylation one obtains a surface concentration of attached diols of about 2.5 groups/nm², which is approximately 1.5 times higher than that resulting from the combustion analysis.

On the one hand, the tosylation of alcohol-substituted silicas with TsCl gives good activated sorbents containing very reactive tosylate groups

Table 1
Epoxidation of vinylsilica

Time of reaction (h)	Concentration of epoxy groups obtained from titration with Na ₂ S ₂ O ₃ (groups/nm ²)		
	Acetic acid + 13% H ₂ O ₂	Acetic anhydride + 13% H ₂ O ₂	Acetic anhydride + 25% H ₂ O ₂
2	–	–	2.20
6	0	0.80	–
20	–	–	1.55
24	0	0.22	–
29	0	0.17	–
48	–	–	0.22

Table 2
 Characteristics of tosyl- and haloalkyl-activated silicas

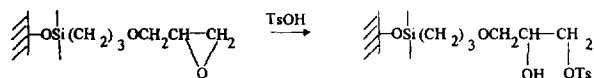
Functional groups of the activated silica	Concentration of attached groups (groups/nm ²)		Average distance between active groups ^a (nm)
	Obtained from %C	Obtained from titration	
$\begin{array}{cc} \text{---CH---CH} \\ \quad \\ \text{OTs} \quad \text{OTs} \\ \text{(according to Eq. 4)} \end{array}$	1.8	1.25 ^b 2.50 ^c	0.95
$\begin{array}{cc} \text{---CH---CH} \\ \quad \\ \text{OTs} \quad \text{OTs} \\ \text{(according to Eq. 5)} \end{array}$	1.8	0.64	1.34
$-(\text{CH}_2)_{11}\text{OTs}$	1.6	1.33	0.97
$-\text{CH}_2\text{Br}$	2.1	2.15	0.74
$-(\text{CH}_2)_3\text{Br}$	2.2	2.20	0.72
$-(\text{CH}_2)_5\text{Br}$	2.5	2.14	0.74
$-(\text{CH}_2)_{11}\text{Cl}$	1.7	1.65	0.84

^a Calculated from: $d_{av} = 1.075/\sqrt{c}$ [3]; c (groups/nm²) was taken from titrimetric data.

^b Assuming tosylation of both hydroxyl groups.

^c Assuming tosylation of one hydroxy group.

attached in a high concentration. On the other hand, the tosylation procedure discussed under Experimental is time consuming and requires the use of corrosive, toxic and carcinogenic reagents. We therefore studied a different method for the tosylation of silica, namely the reaction between an epoxy-activated silica and *p*-toluenesulphonic acid:



The results obtained are given in Table 2 and show that the reaction does take place and tosylated silica is obtained, as follows from both titrimetric and combustion analysis. Only a low concentration of attached tosyl groups is achieved. We consider that this is because TsOH reacts only with epoxides and therefore the concentration of tosyl groups cannot exceed the concentration of the initial epoxy groups (see Table 2).

3.3. Haloalkylsilica

This type of sorbent was obtained via the reaction [1,3,6]



Initial silanes can be obtained via the hydrosilylation of haloalkenes [16,24] or (for $n = 1$) through the direct halogenation of methylsilanes [15].

The bonding density of haloalkyl groups is the highest among the sorbents studied (Table 2) and is close to that theoretically possible for organosilanes on a silica surface (2.3–2.4 group/nm² [1,6,24]). Therefore, the average distance between the attached functional groups is the least among the silicas modified with organosilicon compounds. Hence the haloalkyl-activated silicas seem to be very attractive for the achievement of sorbents with a high ligand

loading. Using these activated silicas, a number of ion-exchange and ligand-exchange stationary phases with high ligand loadings (up to 2.2 groups/nm²) have been obtained [10,11,25–28].

It should also be noted that bromoalkyl-activated silica has advantages over chloro- and iodoalkyl-activated silicas. Iodoalkyl compounds are less readily available and less stable than bromoalkyl compounds [13]. Chloroalkyl-activated silicas, have been shown to be less active than bromoalkyl-activated silica [1,6]. Further, immobilization of any species on chloroalkyl-activated silicas produces Cl⁻ ion, which is considered to cause the destruction of Si–O–Si bonds in the bonded layer [6,29,30].

A distinguishing feature of haloalkyl-activated silicas is a poly-site attachment of polyfunctional ligands. In our previous studies [31–33] we examined the immobilization of the oligopeptides bacitracin A and gramicidin S, which contain two free amino groups per molecule, i.e., they are bifunctional ligands. We found that for the haloalkyl-activated silicas the proportion of one-site ligand attachment is the lowest and can reach even zero (see Table 3).

Poor wettability with aqueous solutions, especially for long-chain modifiers, is usually consid-

ered as a drawback of haloalkylsilicas. Also, these sorbents are wrongly believed to show considerable non-specific sorption of biological species. However, we have succeeded in the affinity purification of proteinases on haloalkyl-activated silicas which contain attached bacitracin and gramicidin as the affinity ligands [31–33]. We found that non-specific sorption of proteinases does not occur, even for sorbents with a C₁₁ spacer, after the treatment of such sorbents with a Tris buffer solution.

4. Conclusions

In our opinion, the haloalkylsilicas are the best activated matrices for the preparation of many chromatographic sorbents. These activated silicas can be obtained via a one-stage synthesis and have virtually the maximum possible bonding density of attached active groups (up to 2.2 groups/nm²). Such sorbents appear feasible for producing a dense layer of attached ligands and/or poly-site attachment of polyfunctional ligands.

A simple method for the preparation of a tosyl-activated silica without the use of TsCl is suggested. The method involves the treatment of

Table 3
Proportion of one-site binding for the ligands bacitracin A and gramicidin S attached to the activated silicas

Type of active group	Ligand	Proportion of one-site binding (%)
$\begin{array}{c} \text{—CH—CH} \\ \quad \\ \text{OTs} \quad \text{OTs} \end{array} \quad 2$	Bacitracin A	23 ± 3
$\begin{array}{c} \text{—(CH}_2\text{)}_3\text{Br} \\ \text{—(CH}_2\text{)}_{11}\text{OTs} \end{array}$	Bacitracin A Bacitracin A	0 22 ± 3
$\begin{array}{c} \text{—CH—CH}_2 \\ \diagdown \quad / \\ \text{O} \end{array}$	Bacitracin A	96 ± 12
$\begin{array}{c} \text{—CH—CH}_2 \\ \diagdown \quad / \\ \text{O} \end{array}$	Gramicidin S	110 ± 14
$\begin{array}{c} \text{—CH}_2\text{Br} \\ \text{—(CH}_2\text{)}_3\text{Br} \end{array}$	Gramicidin S Gramicidin S	62 ± 8 54 ± 7

epoxy-activated silicas with TsOH. This method is recommended if not a very high concentration of active groups is acceptable.

A method for the preparation of epoxy-activated silicas is proposed. The method involves the treatment of attached alkenes with epoxidizing mixtures. This method allows high concentrations of attached epoxides to be obtained (up to 2.2 groups/nm²).

The epoxy-activated sorbents obtained with the use of GOPTAS seem attractive for the preparation of isolated attached ligands and/or the one-site attachment of polyfunctional ligands.

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