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Effect of calcium-modified silica on retention and selectivity in normal-phase liquid chromatography

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Abstract

Modification of a silica surface by calcium ions was studied by high-performance liquid chromatography. After treatment at pH 7.0 and 9.0 with calcium hydroxide, the calcium-modified silicas were subjected to physical and chemical analysis. From calcium measurement with a flame atomic absorption spectrometer, the extent of calcium adsorption was determined as 650 ppm on calcium-modified silica at pH 7.0 and 4800 ppm on calcium-modified silica at pH 9.0. The amount of calcium on the original silica was 58 ppm. The separation factor (α) for N-methylaniline versus N,N-dimethylaniline was measured to be 5.1 on calcium-modified silica at pH 9.0, but could not measured on calcium-modified silica at pH 7.0 or the original silica, using methanol–*n*-hexane (1:99, v/v) as the eluent. By comparison of calcium and amino-modified silica, the separation factors were measured under the same HPLC conditions and was found to be 9.0 on amino-modified silica. The results show that calcium-modified silicas are able to separate some basic model compounds as well as amino-modified silica.

1. Introduction

The surface of silica and adsorption on it have been the subject of many investigations. It has been shown that specific adsorption occurs on the surface silanol groups [1,2]. On the other hand, as the acidic silanol groups are responsible for the cation-exchange properties of silica, the theoretical specific capacity, Q_0, in aqueous solution was shown to be equivalent to the concentration of surface hydroxy groups, which is about 8 μmol/m^2 for a totally hydroxylated silica [3]. For strongly hydrated multivalent cations, the sorption capacities enhanced considerably on increasing the pH, as was shown by Vydra and co-workers [4,5].

Depending on pH, metal ions, for example, may exist in their non-hydrated or their hydrated form. As a consequence of the above considerations, one can expect a very complex ion-exchange behaviour of silica in electrolyte solution. Whereas water and aqueous solutions reduce the activity and acidity of the surface sites of silica, these properties are considerably enhanced in anhydrous media. In the special case of silica catalysts used for various heterogeneous reactions, such as hydration–dehydration and isomerization, the acidity can be measured with the aid of Hammett and arylcarbinol indicators by titration with amines in organic solvents [6].

On the other hand, flash chromatography [7]

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provides a rapid and inexpensive general method for the preparative separation of mixtures requiring only moderate resolution. However, there are a few reports on the effect of silica surface modification by calcium ions on the chromatographic properties of the packing [8–15]. Therefore, we have studied the preparation and evaluation of inexpensive calcium-modified silica for the large-scale preparative separation and routine purification of organic compounds, in comparison with the expensive high-performance liquid chromatographic (HPLC) column gels.

2. Experimental

2.1. Reagent and materials

Benzene, N,N-dimethylaniline, N-methylaniline, 2-ethylpyridine, aniline, pyridine, dimethylphthalate, di-*n*-butyl phthalate, phenol and calcium hydroxide were obtained from Wako (Osaka, Japan). The other reagents and organic solvents were of analytical-reagent grade.

2.2. Porous silicas

Porous silica were prepared in our laboratories (Table 1, Ca-0). The particle size was 9.8 μm.

2.3. Calcium hydroxide solution

Calcium hydroxide solution (1 g per 100 ml) was prepared by dissolving calcium hydroxide in distilled, deionized water. After standing for 24 h at room temperature, the calcium hydroxide solution was filtered with a membrane filter (0.5 μm).

2.4. Calcium-modified silica

A 10-g amount of dried silica (original, Ca-0, Table 1) was immersed in 100 ml of distilled, deionized water. The silica suspension was then carefully adjusted to pH 7.0 (4.8 ml of the calcium hydroxide solution were added), boiled for 2 min, filtered with a membrane filter (0.5 μm), washed several times with methanol and dried in vacuo at 70°C for 1 day, finally producing Ca-I-modified silica, the properties of which are given in Table 1.

Also, the silica suspension was adjusted to pH 9.0 (78.6 ml of the calcium hydroxide solution were added), and the same procedure as with the Ca-I-modified silica series in Table 1 was then carried out, producing Ca-II-modified silica (Table 1).

The calcium contents of the Ca-0, Ca-I and Ca-II materials were determined with a Seiko (Tokyo, Japan) SAS-727 atomic absorption spectrometer, giving the data in Table 1. The specific surface areas were determined with a Shibata SA-1000 surface area pore volume analyser and are also given in Table 1. The conductivities were measured for suspensions of Ca-0, Ca-I or Ca-II, but not for solid material. The concentrations of the suspensions (5% slurry of Ca-0, Ca-I or Ca-II) were determined with a Kyoto Electronics (Kyoto, Japan) CM-117 conductivity meter, giving the data in Table 1.

The pK_a values of the surface sites were measured with the aid of Hammett and arylcarbinol indicators by titration with amines in or-

Table 1
Characteristics of calcium-modified silica

Modified silica	Specific surface area (m^2/g)	Mean pore diameter (Å)	Pore volume (ml/g)	pH[a]	Conductivity[a] (μS)	Calcium adsorption* (ppm)	Surface acidity (pK_a)
Ca-0 (original)	279	176	1.23	5.8	5	58	3.3
Ca-I	274	184	1.26	6.6	96	650	4.0
Ca-II	252	174	1.22	8.9	168	4800	4.8

[a] 5% Slurry solution.

Table 2
Chromatographic data for di-*n*-butyl phthalate (DBP) and dimethyl phthalate (DMP) (as neutral model compounds) on Ca-0-, Ca-I-, Ca-II- and NH_2-modified silica

Modified silica	Separation factor, $\alpha = k'_A/k'_B$ (DMP vs. DBP)
Ca-0	2.94
Ca-I	2.86
Ca-II	2.79
NH_2	2.53

Eluent, methanol–*n*-hexane (1:99, v/v); other conditions as in Fig. 1.

ganic solvents. The results are given in Table 1 [8].

2.5. Stability of distilled water-washed Ca-II-modified silica

Ca-II-modified silica was washed off the column with distilled water for 1000 min at 1 ml/min, finally producing Ca-III-modified silica.

2.6. Apparatus

The HPLC measurements were carried out on a Twincle instrument (Jasco, Tokyo, Japan), equipped with a Uvidec-100 IV variable-wavelength detector (Jasco) and a column of 250 × 4.6 mm I.D., packed with Ca-0-, Ca-I-, Ca-II- and Ca-III-modified silica.

3. Results and discussion

As shown in Table 2, the model neutral compounds could be separated on Ca-0-, Ca-I- and Ca-II-modified silica as well as NH_2-modified silica [16,17] with the same eluent [methanol–*n*-hexane (1:99, v/v)]. Table 2 shows the separation of dimethyl phthalate versus dibutyl phthalate. Owing to the influence of calcium no separation effect was seen on Ca-0-, Ca-I- and Ca-II-modified silica.

Fig. 1 shows the chromatographic behaviour of N,N-dimethylaniline and N-methylaniline, as basic model compounds, compared with NH_2-modified silica. As can be seen, N,N-dimethylaniline and N-methylaniline could be separated on Ca-II-modified silica, and also NH_2-modified silica, but not on Ca-0- and Ca-I-modified silica with the same eluent [methanol–*n*-hexane (1:99, v/v)]. It was presumed that the effect of ion-exchange sorption on Ca-II controlled the separation effect in the presence of calcium ions.

The number of theoretical plates (N) and the peak asymmetry factor (A_s) for basic model

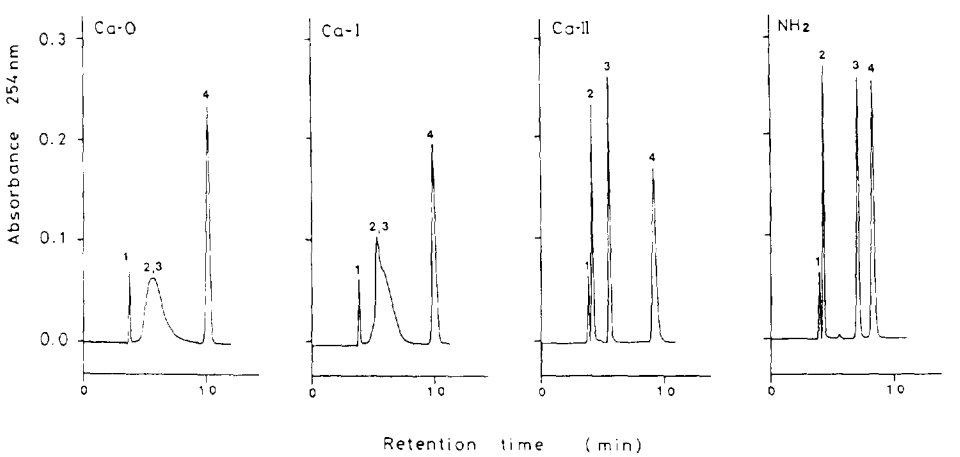

Fig. 1. Chromatographic behaviour of N,N-dimethylaniline and N-methylaniline (as basic model compounds) on Ca-0-, Ca-I- and Ca-II-modified silica, compared with NH_2-modified silica. Column, 250 × 4.6 mm I.D.; eluent, methanol–*n*-hexane (1:99, v/v); flow-rate, 1.0 ml/min; detection, UV at 254 nm. Peaks: 1 = benzene; 2 = N,N-dimethylaniline; 3 = N-methylaniline; 4 = dimethyl phthalate.

compounds (N,N-dimethylaniline, N-methylaniline, 2-ethylpyridine, aniline and pyridine) were measured on Ca-0-, Ca-I- and Ca-II-modified silica, with the same eluent as above. As the amount of calcium on the modified silica increases from Ca-0 to Ca-I to Ca-II, N and A_s can be expected to increase dramatically, as shown in Table 3 and Fig. 1.

The separation factors, α, were measured under the same HPLC conditions using Ca-0-, Ca-I- and Ca-II-modified silicas in comparison with NH_2-modified silica. Table 4 shows that the separation of phenol vs. dimethyl phthalate, owing to the influence of calcium ions, was worse on Ca-II- and NH_2-modified silica than on Ca-0- and Ca-I-modified silica.

Fig. 2 shows the relationship between the amount of calcium adsorbed (ppm) on Ca-0 and the calcium equilibrium concentration (ppm), as the calcium adsorption isotherms for silica at 25°C, using batch adsorption experiments. It can be seen that the extent of calcium adsorption on Ca-0 increases on elevating the calcium equilibrium concentration (ppm).

By increasing the extent of calcium adsorption, the conductivity (S) and pH, basic model compounds could be separated on Ca-0-, Ca-I- and Ca-II-modified silica (Tables 1 and 3 and Fig. 1). Fig. 3 shows the relationship between the extent of calcium adsorption on Ca-0 from a 5% slurry solution for each silica treated at different pH values. As can be seen, the extent of calcium adsorption increases as the pH of treatment increases.

Table 5 shows the separation factors, α, for basic model compounds (N,N-dimethylaniline

Table 4
Chromatographic data for phenol (as acidic model compound) on Ca-0-, Ca-I-, Ca-II- and NH_2-modified silica

Modified silica	Separation factor, $\alpha = k'_A/k'_B$ (phenol vs. DMP)
Ca-0	3.06
Ca-I	2.86
Ca-II	–
NH_2	–

DMP = dimethyl phthalate. Other conditions as in Fig. 1.

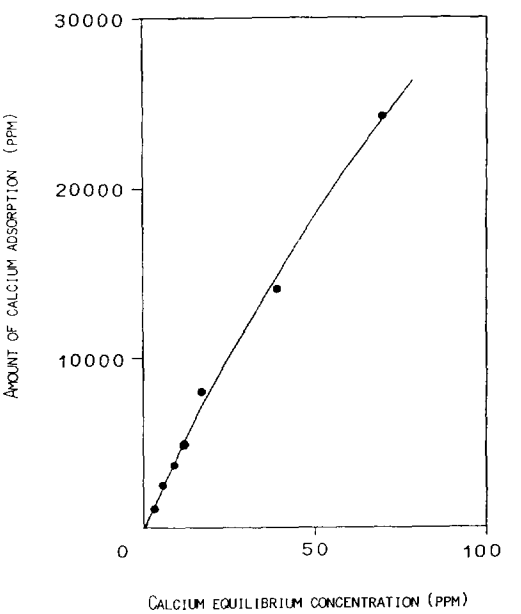

Fig. 2. Relationship between the extent of calcium adsorption and the calcium equilibrium concentration, as calcium adsorption isotherms for silica at 25°C.

Table 3
Capacity factor (k'), number of theoretical plates (N) and peak asymmetry factor (A_s) on Ca-0-, Ca-I- and Ca-II-modified silica

Modified silica	N,N-Dimethylaniline			N-Methylaniline			2-Ethylpyridine			Aniline			Pyridine		
	k'	N	A_s	k'	N	A_s	k'	N	A_s	k'	N	A_s	k'	N	A_s
Ca-0	0.13	4190	2.5	0.61	2516	5.4	1.93	152	21.3	2.49	1888	9.8	3.57	342	11.0
Ca-I	0.10	4962	2.0	0.58	3442	3.7	1.86	189	26.1	2.39	2874	5.7	3.56	487	20.1
Ca-II	0.02	4884	1.2	0.42	5784	1.3	0.83	5780	1.5	1.30	6310	1.4	1.61	4805	1.7
Ca-III	0.07	4410	1.2	0.50	5507	1.4	1.19	3150	2.1	2.62	6202	1.3	3.00	1070	4.0

N: per 25 cm. Other conditions as in Fig. 1.

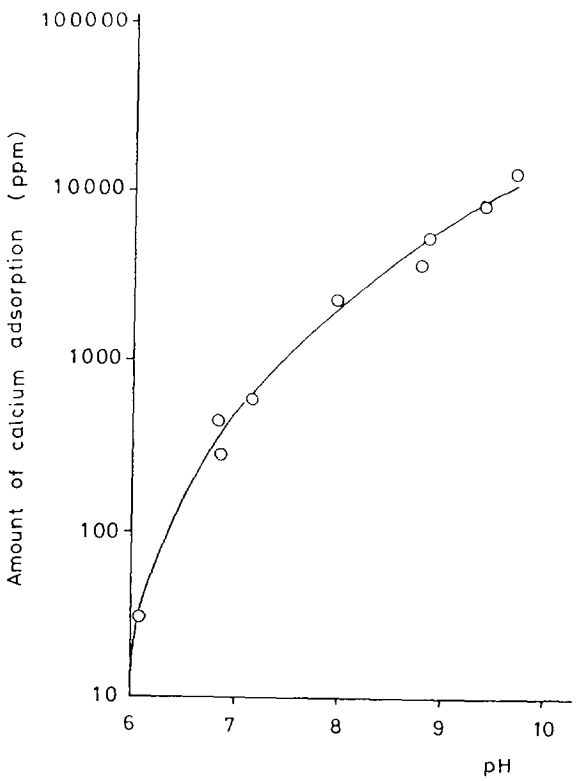

Fig. 3. Relationship between the extent of calcium adsorption from a 5% slurry solution for silica treated at different pH values.

and N-methylaniline) using NH_2-modified silica. As shown in Table 5 and Fig. 1 (Ca-II), Ca-II-modified silica could be also separate some basic model compounds as well as NH_2-modified silica.

Fig. 4 shows comparative chromatograms of some basic model compounds on Ca-II- and Ca-III-modified silica. It is interesting that the Ca-III-modified silica washed with distilled water could also separate some basic model compounds, as well as non-washed Ca-II-modified silica. The stabilities of the calcium columns were obtained under the conditions used in this study. N and A_s were measured on Ca-II- and Ca-III-modified silica, giving the data in Table 3.

Table 5
Chromatographic data for N,N-dimethylaniline and N-methylaniline (as basic model compounds) on Ca-0-, Ca-I-, Ca-II- and NH_2-modified silica

Modified silica	Separation factor, $\alpha = k'_A/k'_B$ (N-methylaniline vs. N,N-dimethylaniline)
Ca-0	1.00
Ca-I	1.25
Ca-II	5.62
NH_2	9.75

Conditions as in Table 4.

It is suggested that the separation mechanism is due mainly to Ca–Si bonds on the silica surface and that the silanol groups on the silica surface undergo an ion-adsorption interaction with the retention of some basic model compounds.

From studies on liquid chromatography with silica as sorbent, it is generally known that the interaction of the silica surface with the solute depends on the silanol groups. In previous papers [18,19], we assumed that the hydrogen-bonded silanol groups on the silica surface have an inhibitory effect on the retention of the solute and that the retention effect was due mainly to free silanol groups on the silica surface. Moreover, we assumed that the silanol groups which reacted with chemical reagents [hexamethyldisilazane (HMDS) or octadecyldimethylchlorosilane (ODS)], were mainly the free silanol groups on the silica surface. It was also shown that the silanol group concentration, $\alpha_{OH(s)}$, of the reactive silanols is almost constant at about 2 groups per 100 $Å^2$ (1 nm^2, mean pore diameter 116 Å, specific surface area 298 m^2/g, pore volume 1.22 ml/g, mean particle size 5.0 μm). According to our methods [18,19], the amount of calcium on the calcium-modified silica was suggested to be 0.574 groups per 100 $Å^2$ (1 nm^2) at pH 9.0.

These experimental results showed that calcium-modified silicas resulted in a drastic improvement in the separation of basic compounds, and a relatively small amount of calcium was needed to improve the separation of basic compounds.

In conclusion, the inexpensive Ca-II-modified silica seem to be more suitable for large-scale preparative separations of some basic model compounds, with easy preparation of the gels,

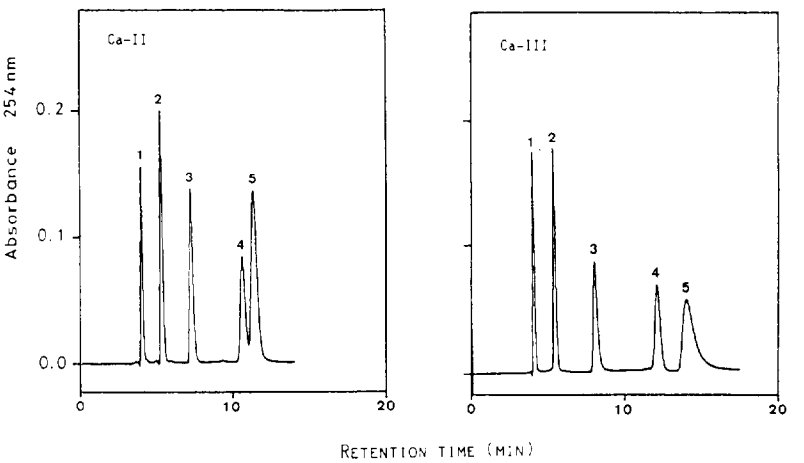

Fig. 4. Stability of calcium modified silica columns washed with distilled water. Other conditions as in Fig. 1. Ca-II = Ca-II-modified silica column not washed with distilled water; Ca-III = Ca-III-modified silica column washed with distilled water (1 ml/min, 1000 min). Eluent: Peaks; 1 = N,N-dimethylaniline; 2 = N-methylaniline; 3 = 2-ethylpridine; 4 = aniline; 5 = pyridine. methanol–*n*-hexane (1:99, v/v).

and health and safety and financial advantages for normal-phase LC packings in the laboratory.

It is concluded from the present investigation that it is not sufficient to evaluate column gels solely with regard to the amount of calcium on the calcium-modified silica. The relationship between the extent of calcium adsorption and the specific surface area of the silica supports must be considered further.

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