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Titania and zirconia: possible new ceramic microparticulates for high-performance liquid chromatography

MASAHIRO KAWAHARA, HIROSHI NAKAMURA* and TERUMI NAKAJIMA
Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, 7–3–1, Hongo, Tokyo 113 (Japan)

ABSTRACT

The adsorptive properties of various new ceramics as column packing materials for high-performance liquid chromatography were evaluated by a newly developed method. Screening of many new ceramics revealed that titania and zirconia have much greater adsorption capacities than silica gel. The adsorptive properties of titania and zirconia columns were found to be different from those of silica gel in chromatography. Further, titania and zirconia proved to have a high resistance to both alkaline and acidic eluents. Applications of these new ceramics columns to the separation of biogenic substances were also studied.

INTRODUCTION

Silica gel and its derivatives have been the predominant column packing materials in modern liquid chromatography, owing to their high resolution[1]. However, their use is limited to the pH range 2.5–7.5 in view of the chemical stability of the silica gel matrices[2]. Recently, new ceramics have been introduced for diverse purposes, *e.g.*, as heat-resistant materials, sensor elements and mechanical parts, owing to their ability to withstand heat, mechanical shock and the effects of various chemicals[3]. The advantageous characteristics of the new ceramics indicate that they should also be usable as packing materials for high-performance liquid chromatography (HPLC). We previously evaluated the adsorptive properties of some new ceramics by determining their adsorption isotherms[4], which indicated that titania and zirconia are promising column packing materials. Therefore, columns of these two new ceramics were investigated for their separating abilities in adsorption chromatography, and applied to the separation of some biogenic substances. In this paper, the separation of ribonucleosides and deoxyribonucleosides on titania and zirconia columns are described.

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EXPERIMENTAL

Materials

Reagents and solvents were of analytical-reagent grade. Silica gel (LiChrosorb Si 60, particle size 5 μm) was purchased from E. Merck (Darmstadt, F.R.G.). Zirconia (non-porous, mean particle size 3 μm) and titania (rutyl type, 1–2 μm) were purchased from Soekawa Chemical (Tokyo, Japan). Titania microbeads (anatase type, porous, pore volume 0.3 ml/g, surface area 45 m^2/g, particle size 5–15 μm) were kindly donated by Catalysts & Chemicals Industries (Tokyo, Japan). Ribonucleosides (adenosine, guanosine, cytidine and uridine) and deoxyribonucleosides (thymidine, 2′-deoxyadenosine, 2′-deoxyguanosine, 2′-deoxycytidine and 2′-deoxyuridine) were purchased from Sigma (St. Louis, MO, U.S.A.).

Measurement of adsorption isotherms

The adsorption isotherms for *p*-hydroxybenzoic acid (*p*-HBA) in hexane–2-propanol (9:1) on the various ceramics were determined by the shake-flask method[4].

HPLC system

A Trirotar-VI (Jasco, Tokyo, Japan) HPLC system was used. The column packing materials were suspended in glycerol–methanol (1:1, v/v) and packed in a stainless-steel tube (100 mm × 4 mm I.D.) by the slurry packing method at a constant pressure of 450 kg/cm^2.

Endurance test

A 100-mg amount of each ceramic, silica gel (LiChrosorb Si 60, 5 μm), titania (microbeads, 5–15 μm) and zirconia (3 μm), was placed in a test-tube and 10 ml of either 1 or 0.1 *M* hydrochloric acid or 50 m*M* or 0.5 *M* sodium hydroxide solution were added. After the tube had been shaken at 40°C for 48 h, the solvent was removed by centrifuging at 1000 *g* for 10 min. The resulting residue was washed with distilled water followed by a similar centrifugation. After the ceramic material had been dried at 110°C for 2 h, it was weighed.

RESULTS AND DISCUSSION

Evaluation of ceramics

The adsorption isotherms of the various ceramics were measured and the amount of *p*-HBA adsorbed per unit weight of the adsorbent (C_S, nmol/mg) and the concentration of *p*-HBA in the supernatant (C_M, μM) were determined. The adsorption isotherms in the liquid phase are commonly represented by either the Henry equation (eqn. 1), the Langmuir equation (eqn. 2) or the Freundrich equation (eqn. 3)[5,6].

$$C_S = K_d C_M \tag{1}$$

where K_d is the distribution constant.

$$C_S = AQ_m C_M/(1 + AC_M) \tag{2}$$

where A is related to the affinity of the adsorbent and Q_m indicates the maximum capacity of the adsorbent. To determine these parameters, following two linearized forms are employed:

$$C_M/C_S = (1/Q_m)C_M + 1/(AQ_m) \quad (2a)$$

$$1/C_S = (1/A)(1/Q_m)(1/C_M) + 1/Q_m \quad (2b)$$

$$C_S = KC_M^{1/n} \quad (3)$$

where K is the capacity of the adsorbent when $C_M = 1$ and n is related to both the adsorption activity and the distribution of adsorption points; a large value of n indicates a higher ratio of the adsorption points with larger heats of adsorption.

Fig. 1 shows the adsorption isotherms on silica gel, titania and zirconia analysed by the four isotherm quations. Table I gives the values of the parameters and the correlation coefficients (r) of these four isotherms. It was found that these isotherms were best fitted by the Freundrich equation (eqn. 3). The Freundrich equation also gave good representation for other ceramics. Therefore, the adsorptive properties of

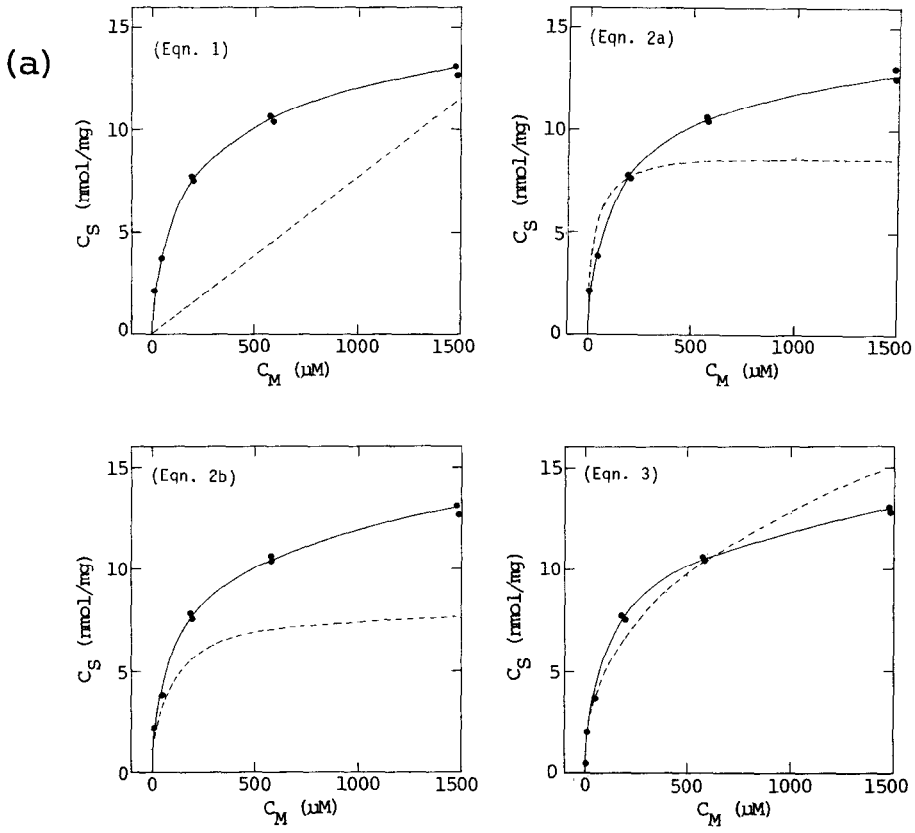

Fig. 1.

(Continued on p. 152)

the ceramics were subsequently determined from the parameters of the Freundrich equation. As shown in Table I, the values of the capacity parameter K for silica gel (LiChrosorb Si 60), titania and ziconia were 0.567, 12.1 and 15.0, respectively, and the values of the activity parameter n were 2.16, 3.79 and 4.14, respectively. These results indicate that titania and zirconia have much larger adsorption capacities than silica gel. The rutyl type of titania showed a smaller adsorption capacity than the anatase type employed in this work.

Further, titania and zirconia were found to possess a high resistance to both alkaline and acidic eluents (Table II). These two new ceramics did not dissolve in either 1 M hydrochloric acid or 0.5 M sodium hydroxide solution on shaking at 40°C for 48 h, whereas silica gel dissolved completely in 0.5 M sodium hydroxide solution.

Separation profiles of titania and zirconia

Titania and zirconia were packed into columns (100 mm × 4 mm I.D.) and their properties in adsorption chromatography were investigated. In Fig. 2, the values of the capacity factor (k') of methyl paraben in eluents with various ratios of hexane and 2-propanol were compared using the three columns. These results indicate that titania and zirconia columns also act as adsorbents in normal-phase chromatography, and

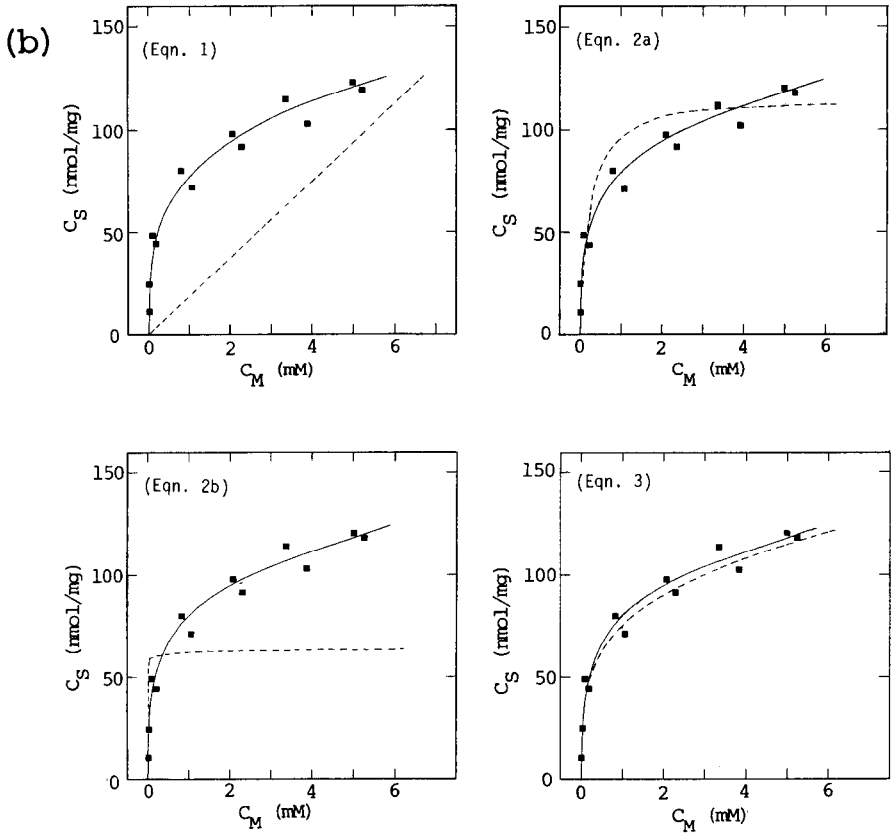

Fig. 1.

that the adsorption was greater on the zirconia column than on the titania and silica gel columns, which is supported by the values of the parameter K in the Freundrich equation. However, the effect of water was severe on the zirconia column and the k' value was found to become small with an eluent containing even a small amount of water.

Fig. 3 shows the separation profiles of parabens with hexane–2-propanol–water (95:4.5:0.5) as eluent on silica gel, titania and zirconia columns. In all instances hexyl, propyl and methyl paraben were eluted in that order. These results suggest that titania and zirconia are actually usable as column packing materials. The recoveries with titania and zirconia seemed to be almost the same as that with silica gel, judging from the peak areas of the solutes. The theoretical plate numbers measured with methyl paraben for silica gel, titania and zirconia were 56 000, 12 000 and 11 000 per metre, respectively. The column efficiencies of titania and zirconia are expected to be improved by using uniform and smaller particle sizes.

The capacity factors (k') of various compounds were compared under the above elution conditions and it was found that the elution order of acidic compounds and

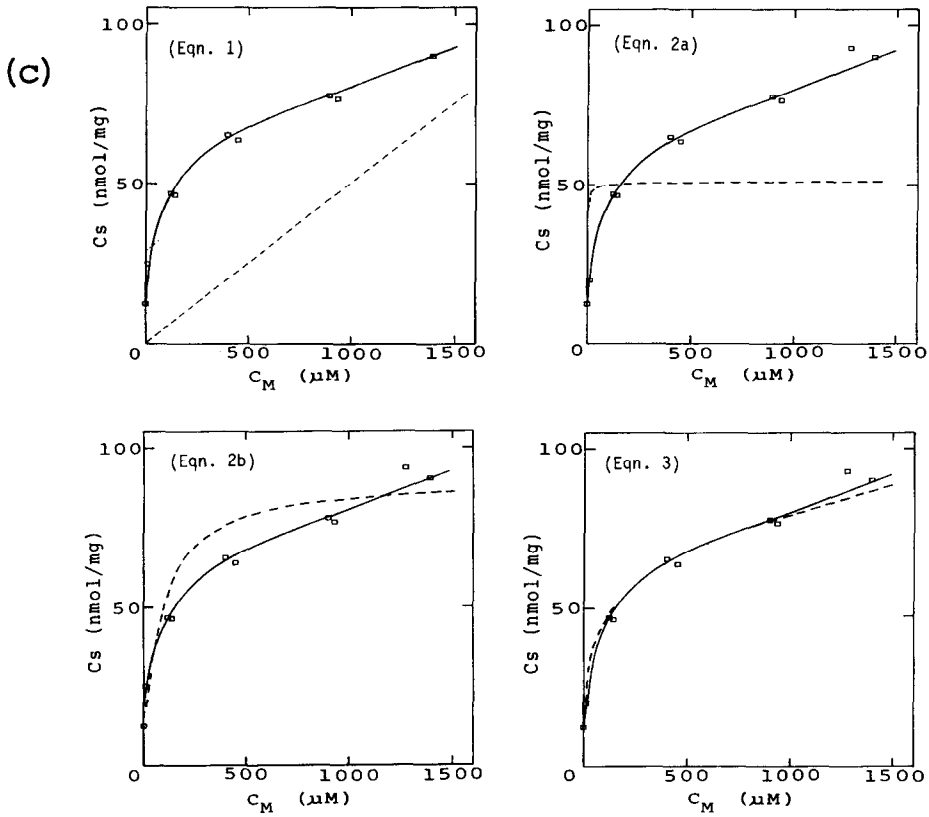

Fig. 1. Adsorption isotherms of (a) silica gel, (b) titania and (c) zirconia as analysed by the four equations. Solid lines, experimental isotherms; dashed lines, theoretical isotherms.

TABLE I

SUMMARY OF PARAMETERS OF ISOTHERM EQUATIONS

Material	*Henry (eqn. 1)*		*Langmuir (eqn. 2a)*			*Langmuir (eqn. 2b)*			*Freundrich (eqn. 3)*		
	K_d *(l/g)*	*r*	*A*	Q_m *(nmol/mg)*	*r*	*A*	Q_m *(nmol/mg)*	*r*	*K*	*n*	*r*
Silica gel	$7.77 \cdot 10^{-3}$	0.874	$3.62 \cdot 10^{-2}$	8.89	0.986	$1.18 \cdot 10^{-2}$	8.04	0.996	$5.67 \cdot 10^{-1}$	2.16	0.980
(Lichrosorb Si 60)											
Titania	$1.88 \cdot 10^{-2}$	0.893	$4.56 \cdot 10^{-3}$	117	0.987	$3.21 \cdot 10^{-1}$	61.3	0.914	12.1	3.79	0.992
Zirconia	$5.17 \cdot 10^{-2}$	0.926	$8.57 \cdot 10^{-1}$	50.8	0.856	$1.26 \cdot 10^{-2}$	90.6	0.989	15.0	4.14	0.997

TABLE II

RESULTS OF ENDURANCE TEST ON NEW CERAMICS

Material	*Recovery (%)*[a]			
	1 M HCl	*0.1 M HCl*	*0.05 M NaOH*	*0.5 M NaOH*
Silica gel	95.5	96.3	73.0	0
Titania	100	99.0	98.2	101
Zirconia	100	99.5	100	101

[a] After shaking in 10 ml of the medium at 40°C for 48 h.

basic compounds was reversed on titania and zirconia columns, *i.e.*, acidic compounds eluted later than basic compounds[7]. This suggests that the adsorption mechanisms with titania and zirconia are different from that with silica gel. This effect should be advantageous in the analysis of basic compounds, which show peak tailing on the usual silica-based columns owing to anionic free silanol groups.

It was possible to use titania and zirconia columns with water-rich eluents such as acetonitrile–water. Table III gives the k' values for various compounds using acetonitrile–water (97.5:2.5) as eluent on silica gel, titania and zirconia columns. The results also indicate that the elution order of pyridine and phenol is reversed on titania and zirconia columns. Further, the k' values of three similar compounds, caffeine, theophylline and theobromine, were very different on the titania and silica gel columns. These unique adsorptive properties of titania and zirconia may be useful in the separation of compounds that are difficult to separate on the usual column packings. Therefore, titania and zirconia columns were applied to the separation of biogenic substances, including nucleosides.

Fig. 4 shows the separation profiles of a mixture of ribonucleosides (uridine,

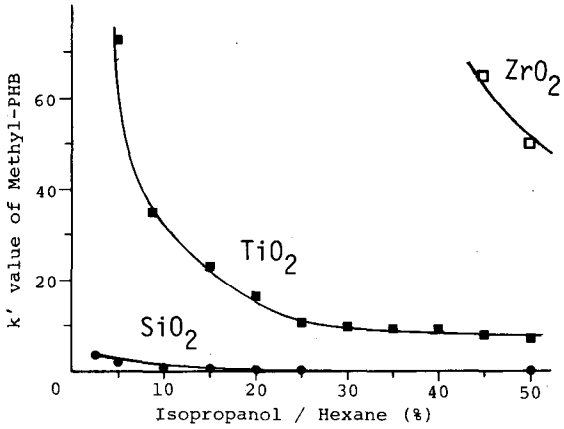

Fig. 2. k' Value of methyl paraben as a function of 2-propanol concentration in hexane (eluent). Column, 100 mm × 4 mm I.D.; column temperature, 40°C; flow-rate, 0.5 ml/min. ● = Silica gel; ■ = titania; □ = zirconia.

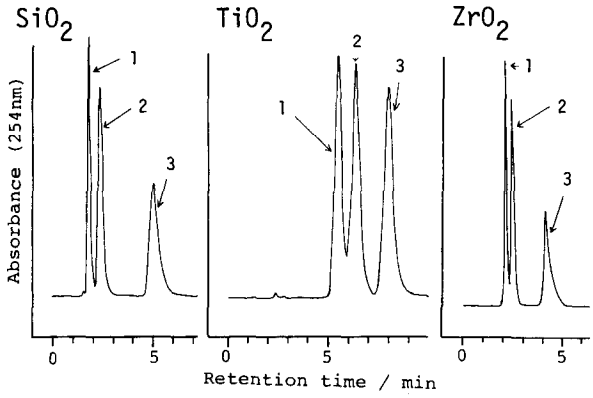

Fig. 3. Chromatograms of parabens on (a) silica gel, (b) titania and (c) zirconia columns. Column 100 mm × 4 mm I.D.; column temperature, 40°C; flow-rate, 0.5 ml/min; eluent, hexane–2-propanol–water (95:4.5:0.5). Peaks: 1 = hexyl paraben; 2 = propyl paraben; 3 = methyl paraben.

guanosine, cytidine and adenosine) and deoxyribonucleosides (thymidine, 2′-deoxyuridine, 2′-deoxycytidine, 2′-deoxyguanosine and 2′-deoxyadenosine) with gradient elution from acetonitrile to 20% acetonitrile on silica gel, titania and zirconia columns. Although, deoxyribonucleosides were eluted rapidly from the titania and zirconia columns as sharp peaks, ribonucleosides were adsorbed strongly and were eluted later as broad peaks. However, ribonucleosides and deoxyribonucleosides eluted at similar retention times on the silica gel column. On the titania column, ribonucleosides and deoxyribonucleosides were also separated with 2 m*M* Tris–HCl buffer (pH 7.0) as the eluent[8]. It is noteworthy that ribonucleosides and deoxyribonucleosides have very similar structures, differing only in the 2′-positions of the sugar moieties, but their behaviours on titania and zirconia are obviously different. This suggests the possibility of chelation of Ti^{IV} or Zr^{IV} to compounds having a vicinal hydroxyl group. Some kinds

TABLE III

k′ VALUES OF VARIOUS COMPOUNDS ON THE THREE COLUMNS

Column, 100 mm × 4 mm I.D.; column temperature, 40°C; eluent, acetonitrile–water (97.5:2.5); flow-rate, 1.0 ml/min.

Compound	*Column*		
	Silica gel	*Titania*	*Zirconia*
Benzene	0.00	0.00	0.00
Phenol	0.03	2.81	0.32
Pyridine	1.00	0.36	0.05
Aniline	0.08	0.10	0.02
Methyl paraben	0.08	12.5	2.39
Caffeine	1.24	0.25	0.05
Theophylline	1.97	>30	2.44
Theobromine	2.56	20.0	11.3

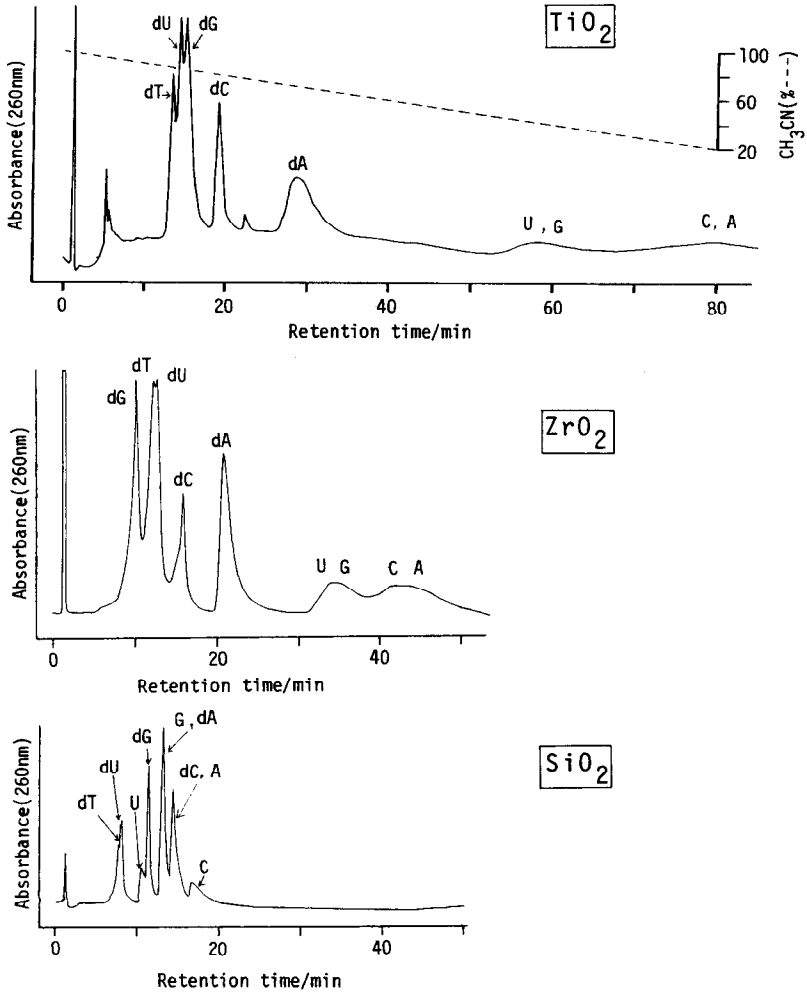

Fig. 4. Chromatograms of nucleosides on (a) titania, (b) zirconia and (c) silica gel columns. Column, 100 mm × 4 mm I.D.; column temperature, 40°C; flow-rate, 1.0 ml/min; eluent A, acetonitrile; eluent B, acetonitrile–water (20:80); elution programme, linear gradient from eluent A to B in 80 min. Peaks: dT = thymidine; dU = 2′-deoxyuridine; dG = 2′-deoxyguanosine; dC = 2′-deoxycytidine; dA = 2′-deoxyadenosine; U = uridine; G = guanosine; C = cytidine; A = adenosine.

of boronate gels[9–11] have been employed both for the group separation of ribonucleosides and deoxyribonucleosides and for the enrichment of compounds having a vicinal hydroxy group. Investigations on the adsorption mechanisms of titania and zirconia are now in progress, including the relationship between crystallographic and chromatographic properties.

In conclusion, new titania and zirconia ceramics have been found to have at least three advantages as column packing materials for modern LC: (1) titania and zirconia possess a high resistance to both alkaline and acidic eluents; (2) they show unique adsorption profiles, adsorb acidic compounds strongly rather than basic compounds;

and (3) they easily permit the separation of compounds such as ribonucleosides and deoxyribonucleosides which are not separable on the usual column packings.

The titania and zirconia columns are also expected to be useful in a two-dimensional HPLC separation system in combination with a different separation mode such as reversed-phase HPLC on ODS-silica. Application of the new ceramics to the separation of various biogenic substances will be reported in the near future.

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