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Titania as a sorbent in normal-phase liquid chromatography[☆]

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Abstract

“Sachtopore” a titanium dioxide sorbent for HPLC is compared to a silica, an alumina and a zirconia sorbent with regard to its physical and chemical properties and examples for chromatographic separations are given. Titania has hydroxyl groups on its surface that are only slightly acidic so that native titania can be used to separate basic molecules under normal-phase conditions. It is shown that this enables the purification of basic fine chemicals and pharmaceuticals. Also, a number of examples for the separation of non-basic isomeric substances mixtures are presented. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Sorbents; Titania; Silica; Alumina; Zirconia; Anilines; Pyridines; Tryptylines

1. Introduction

The most widely used sorbent in high-performance liquid chromatography (HPLC) is silica, SiO₂. Silica is used either as “normal-phase” (NP) material or otherwise with a hydrophobic organic modification of the surface as “reversed-phase” (RP) material. The organic surface modification of silica serves to improve its selectivity particularly in the separation of polar molecules and also to improve the stability of silica, which is inherently poor, especially under basic conditions. Considering the wide scope of inorganic materials known, remarkably few other

sorbents are used in this field of application.

The reason for this probably lies in the difficulty of obtaining inorganic materials with the porous structures required for the purpose. Of the other sorbents only aluminium trioxide (alumina) and zirconium dioxide (zirconia) have gained a humble share of the HPLC-sorbent market. In the meantime, titanium dioxide (titania), of which test samples of different origins have already been described in the literature [1–6], has become commercially available (trade name “Sachtopore”, by Sachtleben, Duisburg, Germany).

In this paper, the properties of Sachtopore (named “titania” from here on) will be described and some comparisons to silica, alumina and (partly) zirconia will be made. It will be shown that the reason for titania’s ability to separate basic sorbates even under normal-phase conditions as well as its high selectivity in the separation of isomers lies mainly in the nature of the Ti–OH groups on its surface.

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2. Materials and methods

2.1. Sorbents

Titania was manufactured by Sachtleben. The other sorbents were selected under the premise that bulk material as well as packed columns were commercially available. The silica sorbent used was LiChrospher Si 60 from Merck, Darmstadt, Germany. The alumina sorbent was Spherisorb Alumina from PhaseSeparations, furnished by Waters, Milford, MA, USA. In the case of zirconia, only a packed column was available from ZirChrom Separations, Anoka, MN, USA.

2.2. Isoelectric points

Isoelectric points (pI) were measured by titration of 2% (v/v) suspensions of the different sorbents in deionised water with 0.1 M HCl and 0.1 M NaOH, respectively, using the electrokinetic sonic amplitude (ESA) apparatus from Matec Instruments, Hopkinton, MA, USA. In the case of zirconia, no ESA measurement was possible because of the lack of a sufficient amount of sample.

2.3. Mean particle sizes

Mean particle size measurements were done with a Fraunhofer diffraction technique using the HELOS apparatus of Sympatec, Clausthal-Zellerfeld, Germany.

2.4. Pore size distribution curves

Pore size distributions were measured with an Autosorb 6 equipped with an Autosorb Degasser Station from Quantachrome, Boynton Beach, FL, USA.

2.5. Scanning electron microscopic images

Scanning electron micrographs were made with

the scanning electron microscope JSM 6400 from Joel, Tokyo, Japan.

2.6. X-Ray diffraction

X-Ray diffraction measurements were carried out with a Diffractometer D500 from Siemens, Karlsruhe, Germany.

3. Results and discussion

3.1. Physical properties of titania

Titania is a crystalline substance with three crystal modifications: brookite, anatase and rutile. Only anatase and rutile are of commercial value, namely as pigments for paints, plastics and synthetic fibres. These crystal modifications are easily distinguished by X-ray diffraction spectroscopy (XRD). Titania with mean pore sizes of 60 Å, 100 Å and 300 Å are pure anatase, whereas the wide pore material with 2000 Å, which is not the topic of this study, is a pure rutile.

Both rutile and anatase have a tetragonal elementary cell. Their crystals consist of an hexagonal array of oxygen atoms in which half of the octahedral spaces are filled by titanium atoms in such a way that each Ti atom is surrounded by six O atoms and every O atom by three Ti atoms. In rutile, the TiO_6 octahedra share two edges whereas in anatase, four edges are shared [7]. Both anatase and rutile have approximately 10 hydroxyl groups per nm^2 of surface area [8].

Like other inorganic HPLC sorbents, titania particles consist of smaller primary particles which are, however, crystalline. Crystallite sizes may be determined according to Debye–Scherrer from the half widths of the XRD peaks. The particles themselves are spherical as shown in Fig. 1. Titania sorbents exhibit a narrow pore size distribution (Fig. 2).

It has already been pointed out by other authors [1–6] that titanium dioxide is virtually insoluble in acids as well as bases, thus enabling separations to be performed at extreme pH values. Its ability to withstand cleaning in place (CIP) procedures with 1 M NaOH is of special interest for preparative HPLC.

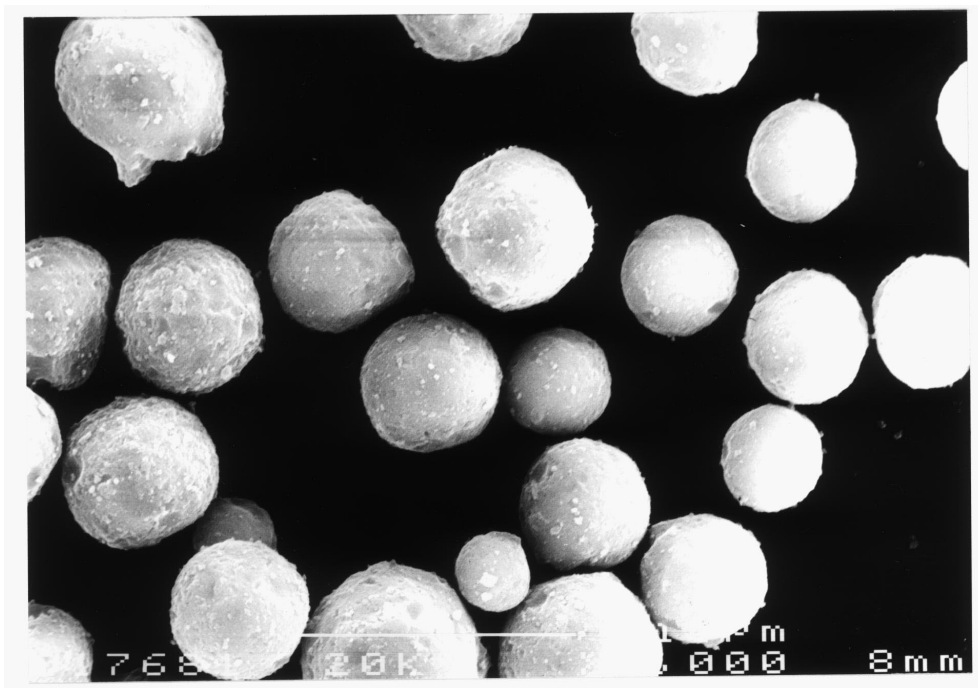


Fig. 1. Scanning electron micrograph of Sachtopore 100 Å, 5 μm.

With its specific gravity of 4 g/cm³ (anatase), titania is denser than silica and alumina sorbents.

3.2. Comparison of physical properties of NP titania, silica, alumina and zirconia

Table 1 compares physical properties of the

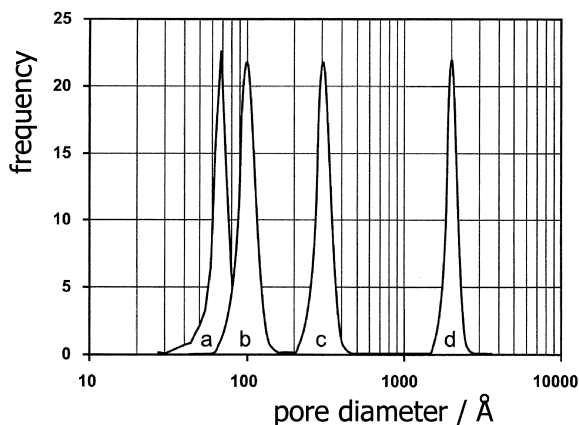


Fig. 2. Pore diameter distributions of Sachtopore. (a) 60 Å, (b) 100 Å, (c) 300 Å, (d) 2000 Å.

sorbents used in this study. It is noteworthy that only silica is completely amorphous with no distinct reflexes in its XRD spectrum (Fig. 3a). It has been pointed out that the degree of crystallinity of silica tends to increase with increasing pore size [6]. This is due to the use of higher temperatures during the production of such a material. Although silica is amorphous, it is generally accepted that in it the coordination number of the Si atoms by oxygen atoms is four [5].

The alumina was partly crystalline (Fig. 3b) with poorly resolved peaks typical for α -Al₂O₃.

In α -Al₂O₃ the oxygen atoms form a hexagonal array in which the Al³⁺ ions are distributed in 2/3 of the octahedral spaces so that each Al is surrounded by six O atoms and each O has four Al atoms as neighbours.

The 100 Å titania of Table 1 is a pure anatase (Fig. 3c).

The crystallinity of the sorbents is related to the specific surface area which may be achieved. Amorphous sorbents have the tendency towards higher specific surface areas. For the production of crystalline oxides an annealing step is required during

Table 1
Comparison of physical properties of sorbents

	SiO ₂	TiO ₂	Al ₂ O ₃	ZrO ₂ ^b
Average particle size (μm)	5.8	5.9	5.2	3.0 ^c
Average pore diameter (Å)	51	112	130	300 ^c
Specific surface area (m ² /g)	660	67	108	–
Pore volume (cm ³ /g)	0.90	0.22	0.30	–
Crystalline modification	Amorphous	Pure anatase	Partly α-Al ₂ O ₃	–
Specific gravity (g/cm ³)	2.2 ^a	4.0	3.5 ^a	5.6–5.9 ^a
Isoelectric point (pH)	2.2	5.6	8.1	–
Specific surface area (m ² /cm ³)	300	16.8	30.9	–
Ratio of void volume to particle volume (%)	66	47	51	–

^a From Ref. [17].

^b Not available as bulk material.

^c Data according to manufacturer.

which levelling and sintering takes place at the surface of the crystals. This leads to lower specific surface areas. It should be pointed out, however, that the crystallinity not only has an influence on the specific surface area itself but also on the homogeneity of the surface. Crystalline materials have orderly structures that extend up to the surface. Since crystalline structures are states with minimum Gibbs free energies, crystal surfaces tend to be formed that are consistent with the surfaces of the elementary lattice cells. Needless to say, for HPLC purposes, it would be favourable to have a high surface area in combination with a high surface homogeneity.

When comparing different sorbents their densities should also be taken into account. Both specific surface areas as well as pore volumes when quoted as mass-based figures (m²/g or cm³/g, respectively) are misleading. Ultimately, the matter of interest in HPLC is, next to selectivity, how many square meters of the sorbent surface are available for the separation in a column (m²/cm³) and what its the ratio of void volume to total volume within it.

For comparison of the sorbents themselves, Table 1 lists the specific surface areas as m² of surface per cm³ of solid sorbent and the porosities of the sorbents as the percentage of void volume within the (spherical) sorbent particles. Comparing titania with silica, it can be seen that the differences in the volume-based specific surface areas are less pronounced than the corresponding mass-based figures. The particle porosities vary between 47% (for titania) and 66% (for silica).

3.3. Surface properties of the sorbents

Adsorption processes on oxide sorbents depend largely upon the properties of the terminating hydroxyl groups on their surfaces which, in turn, are governed by the charge distribution between the metal atom (M), the oxygen atom (O) and the hydrogen atom (H) along the M–O–H groups.

For comparison, three different cases may be distinguished. (a) M^{δ+} OH^{δ-}; (b) M–O–H and (c) MO^{δ-} H^{δ+}.

Which of the three models best represents reality depends upon the electron drawing effect of M. Structure (a) depicts the situation of an M atom with low electron affinity, (b) an intermediate situation leading to a covalent bond along the M–O–H group and (c) the case for an M atom with a high electron drawing potential.

For a given oxide, the electron drawing action of the metal ion increases with increasing positive charge density and decreases with increasing coordination number. A detailed discussion is found in Chapter 6 of Ref. [9].

Of course, the acid base properties of amphoteric oxide surfaces depend upon the polarisation of the surface hydroxyl groups. Table 2 lists ionisation potentials, coordination numbers, ionic radii and the quotient of ionisation potential and coordination numbers as well as the pI values of the sorbents silica, titania and alumina used in this study.

The pI values clearly show that silica has an acidic surface (case c), alumina has a basic surface (case a)

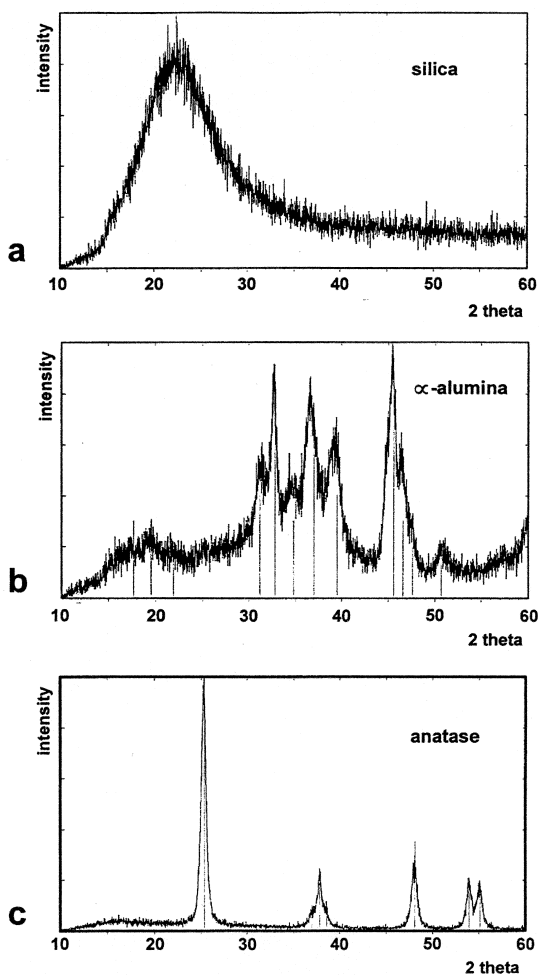


Fig. 3. XRD spectra of (a) silica, (b) alumina and (c) titania. Vertical lines in the spectra of b and c depict expected locations of α -alumina and anatase reflexes.

whereas titania lies in between, presenting slight acidity.

A plot of the pI against the quotient of ionisation potential/coordination number is nearly linear (Fig. 4). Still, it should be kept in mind that pI values from electrophoretic mobility measurements give only information on the “net charge”. Colloidal metal oxide particles themselves may have many different types of hydroxyl groups next to Lewis acid and Lewis base sites [10] and may therefore simultaneously have both positively and negatively charged surface sites under pI measurement conditions. In that case, however, the inhomogenities would effect the chromatographic behaviour. Finally, Table 2 also lists the porosities of the test columns that were determined gravimetrically from columns filled with acetone and carbon tetrachloride, respec-

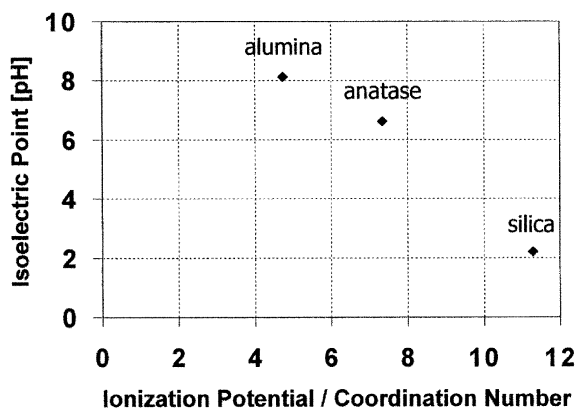


Fig. 4. Plot of pI vs. quotient of ionisation potential and coordination number.

Table 2
Crystallographic data and pI values of sorbents

	SiO ₂	TiO ₂	Al ₂ O ₃	ZrO ₂ **
Ionisation potential (V) ^a	45.14 (Si ⁴⁺)	43.27 (Ti ⁴⁺)	28.45 (Al ³⁺)	34.34 (Zr ⁴⁺)
Coordination number	4	6	6	7
Ion radius (Å)	0.41 (Si ⁴⁺)	0.68 (Ti ⁴⁺)	0.5 (Al ³⁺)	0.8 (Zr ⁴⁺)
Ionisation potential/coordination number (V)	11.29	7.33	4.74	4.83
pI of sorbent (pH)	2.2	6.6	8.1	–
Porosities of test columns (%)	73	74	79	69

^a Ref. [17].

tively. The values range between 69% (zirconia) and 79% (alumina). Silica and titania are almost identical (73% vs. 74%).

3.4. Separation of bases

The weakly acidic surface of titania allows the separation of basic compounds on native TiO_2 . Fig. 5 shows the separation of a mixture of nine different pyridine and aniline derivatives on the titania using *n*-heptane–2-propanol (99.5:0.5, v/v) as an eluent. The peaks are symmetrical and well separated with aniline, the least sterically hindered amine, being retarded longest and leaving the column after 6.75 min. At a flow-rate of $0.75 \text{ cm}^3/\text{min}$ the pressure was comparatively low at 16 bar.

The corresponding initial pressures for silica, alumina and zirconia were, in that order, 37 bar, 25

bar and 43 bar, respectively. It was not possible to separate this mixture under isocratic conditions with these sorbents. Instead, a gradient with an increase of 2-propanol up to 50% within 15 min was run. So, naturally, the pressure increased even more with increasing 2-propanol concentrations. It can be seen (Fig. 6) that alumina separated seven of the nine bases, whereas silica (Fig. 7) and zirconia (Fig. 8) failed because of very strong adsorption.

Alumina gave more or less symmetrical peaks, apart from the last one, which appears to have an underlying additional peak and does show some tailing.

Whereas in the case of silica these results were expected, for alumina and especially zirconia they were surprising in view of published results by Kurganov et al. [6]. However, it is known, that zirconia has a number of differently coordinated zirconium atoms on its surface, one of which is an

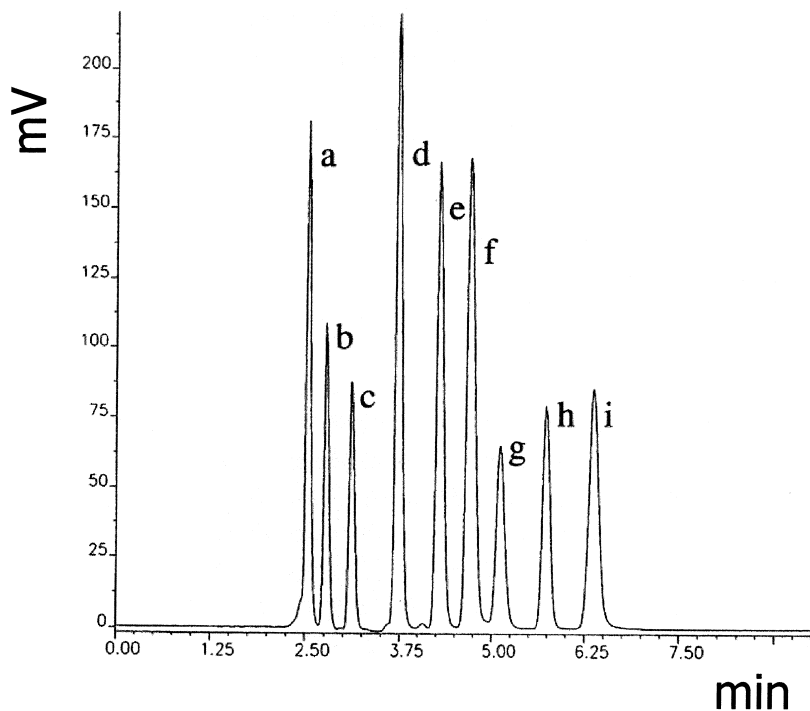


Fig. 5. Separation of substituted aniline and pyridine derivatives on native titania. (a) *N,N*-Dimethylaniline, (b) 2-ethylaniline, (c) *N*-methylaniline, (d) 2,6-dimethylpyridine, (e) 2-methylpyridine, (f) 2,4-dimethylpyridine, (g) pyridine, (h) 4-methylpyridine, (i) aniline. Column: 100 \AA , $5 \mu\text{m}$, $150 \times 4 \text{ mm}$. Eluent: *n*-heptane–2-propanol (99.5:0.5, v/v). Flow: $0.75 \text{ cm}^3/\text{min}$. Detection: UV at 254 nm. Temperature: ambient. Pressure: 16 bar.

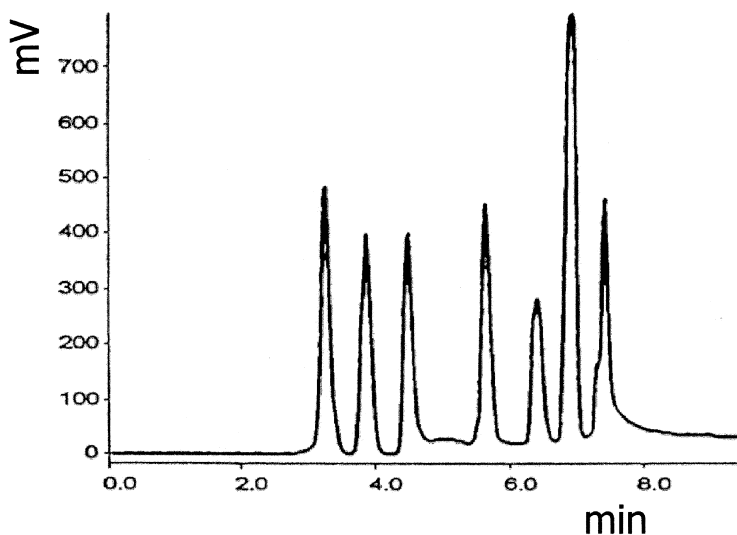


Fig. 6. Separation of substituted aniline and pyridine derivatives on native alumina.

extremely acidic Lewis acid site [11]. The existence of these Zr^{4+} sites with a coordination number of four instead of seven for oxygen was confirmed by the use of paramagnetic nitroxyl ion probes in electron spin resonance (ESR) spectroscopy [12]. Separations of basic molecules can only be accomplished when these sites are masked by other strong Lewis bases such as phosphate ions [11,13] or when other hard bases such as OH^- , F^- or carboxylates are added to the buffer [14,15]. Of course, another

solution is to transform zirconia into a reversed-phase [3] and this seems to be the most effective way [15].

Similarly strong Lewis acid sites were reported to have been found on alumina also [12]. Again, to mask these sites, columns may be equilibrated by alkaline treatment so that bases are absorbed less [16].

To our knowledge, so far there are no reports on the existence or absence of similar Lewis acid sites

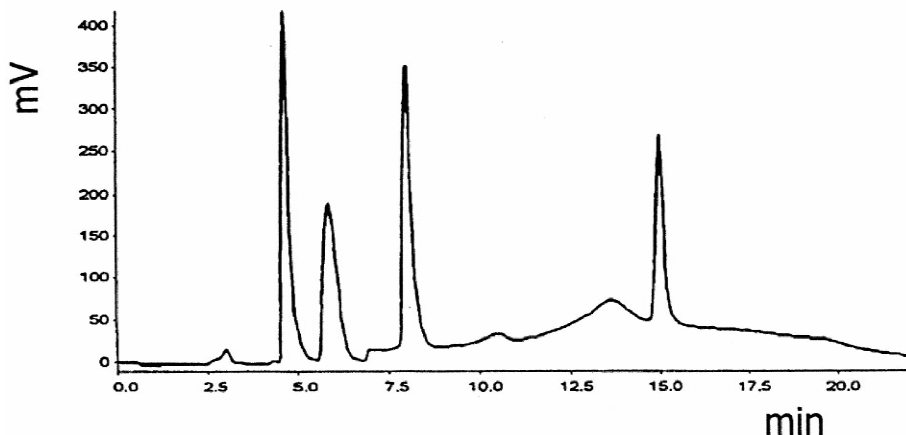


Fig. 7. Separation of substituted aniline and pyridine derivatives on native silica.

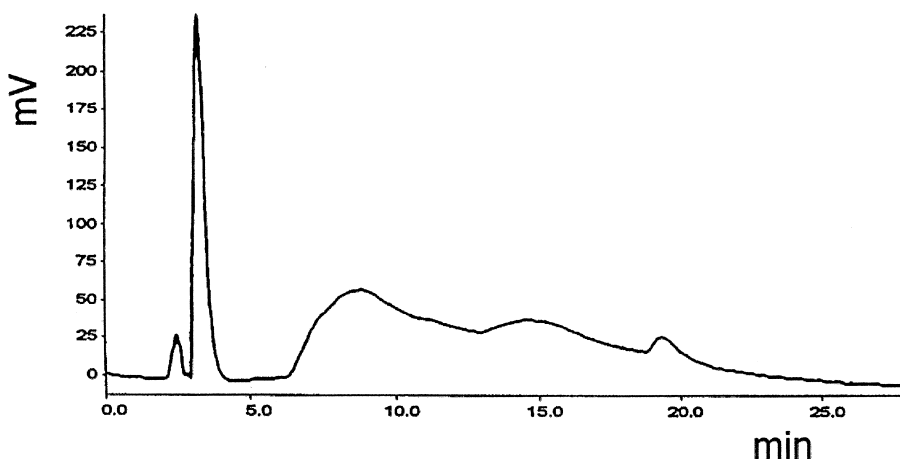


Fig. 8. Separation of substituted aniline and pyridine derivatives on native zirconia.

on titania sorbents for chromatography. This topic is momentarily under investigation in our laboratory.

3.5. Typical behaviour of titania in chromatography; Van Deemter curve

Typical Van Deemter curves of native titania have minimum reduced plate heights of approximately 4.5 particle diameters at a flow-rate of $0.5 \text{ cm}^3/\text{min}$

($250 \times 4 \text{ mm}$ standard column; $5 \mu\text{m}$ titania, 100 \AA pore diameters; chrysene in *n*-heptane).

3.6. Further separations with Sachtopore

Native titania has astonishing capabilities for separating bases and many isomers. Some examples are presented below.

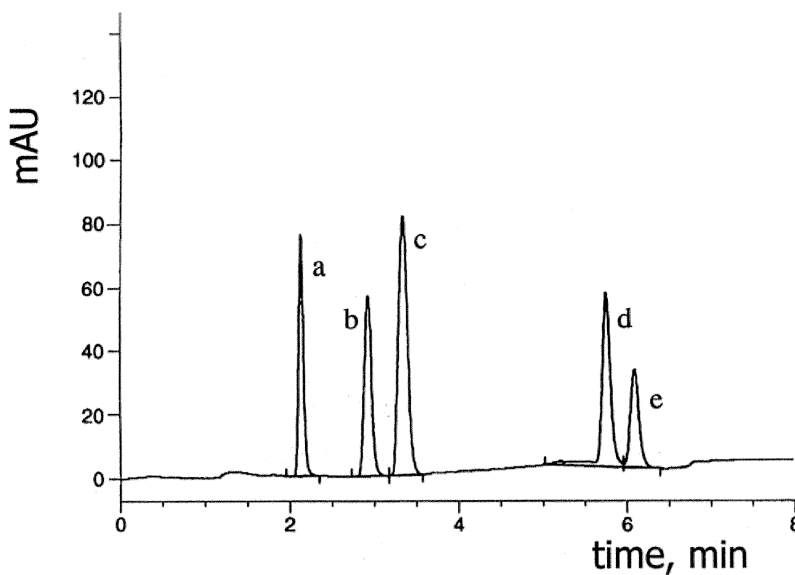


Fig. 9. Separation of mono- and dichloroanilines in a gradient elution on native titania. (a) 2,3-Dichloroaniline, (b) 2-chloroaniline, (c) 2,4-dichloroaniline, (d) 3-chloroaniline, (e) 3,4-dichloroaniline. Column: 100 \AA , $5 \mu\text{m}$, $150 \times 4 \text{ mm}$. Eluent: *n*-heptane–2-propanol (99:1) to (19:81) within 4 min. Flow: $1.5 \text{ cm}^3/\text{min}$. Temperature: 43°C . Detection: UV at 245 nm .

3.6.1. Isomers of chloroaniline

Fig. 9 shows the separation of mono- and dichloroanilines in a gradient elution. This example supplements the separation of aniline and pyridine derivatives of the previous section (Fig. 5). The solutes appear in the expected order and with symmetrical peaks with excellent resolutions.

3.6.2. Basic psychopharmaceuticals (tryptiline derivatives)

Native titania is selective as an adsorbent not only in the separation of basic molecules, but also when it comes to the separation of different isomers of basic reagents. Fig. 10 shows the separation of amitriptyline, a tertiary amine, and noramitriptyline, a secondary amine. Fig. 11 presents the separation of isomers of hydroxynortryptiline. In both cases, symmetrical peaks were obtained using mixtures of dichloroethane and methanol with trifluoroacetic acid (TFA) as a displacer.

3.6.3. Isomers of flumethrine (plant protection chemical)

Fig. 12 shows the chromatogram of a mixture of seven different isomers of flumethrine. The isocratic mobile phase of *n*-heptane–methyl *tert*-butyl ether (MTBE) led to symmetrical elution peaks at moderate retentions of approximately 3.5 min.

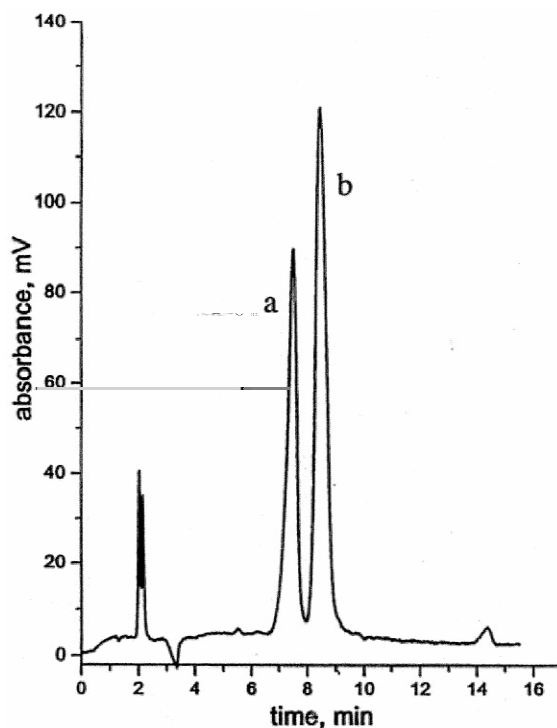


Fig. 11. Separation of diastereomers of hydroxynortryptiline on native titania. (a) *Z*-10-Hydroxynortryptiline, (b) *E*-10-hydroxynortryptiline. Column: 100 Å, 5 μm, 250×4 mm. Eluent: dichloromethane–methanol (TFA) [97.5:2.5 (0.1%), v/v]. Flow: 1 cm³/min. Temperature: ambient. Detection: UV at 254 nm.

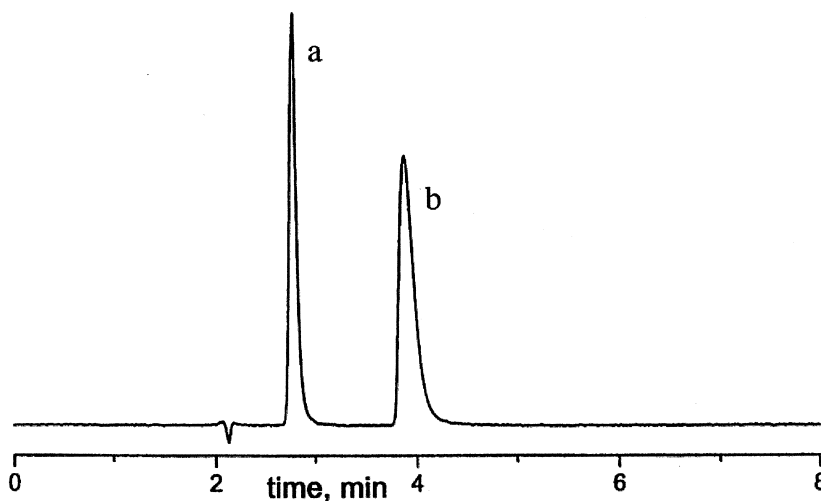


Fig. 10. Separation of amitriptyline and noramitriptyline on native titania. (a) Amitriptyline, (b) noramitriptyline. Column: 100 Å, 5 μm, 150×4 mm. Eluent: dichloromethane–methanol [(TFA) (93:7) (0.2%), v/v]. Flow: 1 cm³/min. Temperature: ambient. Detection: UV at 254 nm.

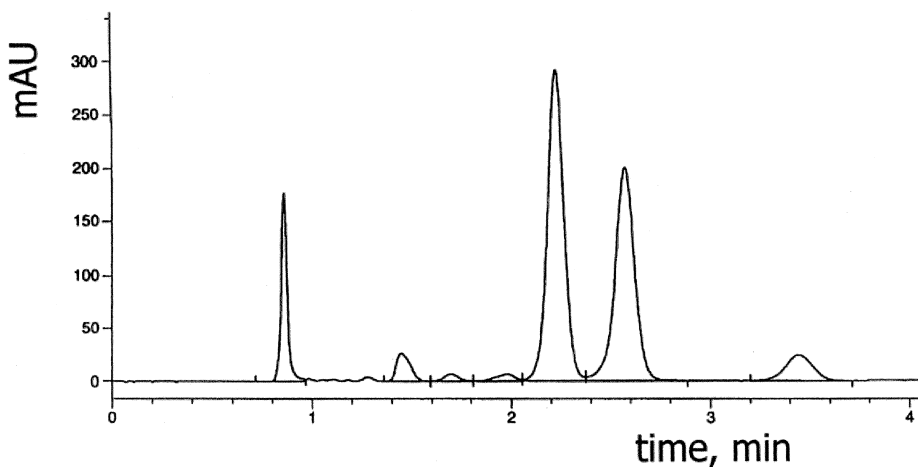


Fig. 12. Separation of a mixture of seven different isomers of flumethrine on native titania. Column: 100 Å, 5 μm, 150×4 mm. Eluent: *n*-heptane–MTBE (60:40, v/v). Flow: 1.5 cm³/min. Temperature: 43°C. Detection: UV at 204 nm.

3.6.4. Isomers of endosulfan (insecticide and acaricide)

Fig. 13 shows the separation of the α- and β-isomers of the insecticide endosulfan. Changing the eluent from 2-propanol–methanol–*n*-heptane (1:20:480, w/w/w) mixture to pure dichloromethane reduced the elution time to less than 3 min because of better solubility of the endosulfans in that solvent.

3.6.5. Glycyrrhetic acid (anti inflammatory pharmaceuticals)

Another example for the selectivity of titania is shown in Fig. 14a and b, chromatograms of a fresh and aged solution of the anti inflammatory reagent glycyrrhetic acid. The product peak appears after approximately 2.5 min under the cited conditions. After 30 days, several other peaks belonging to

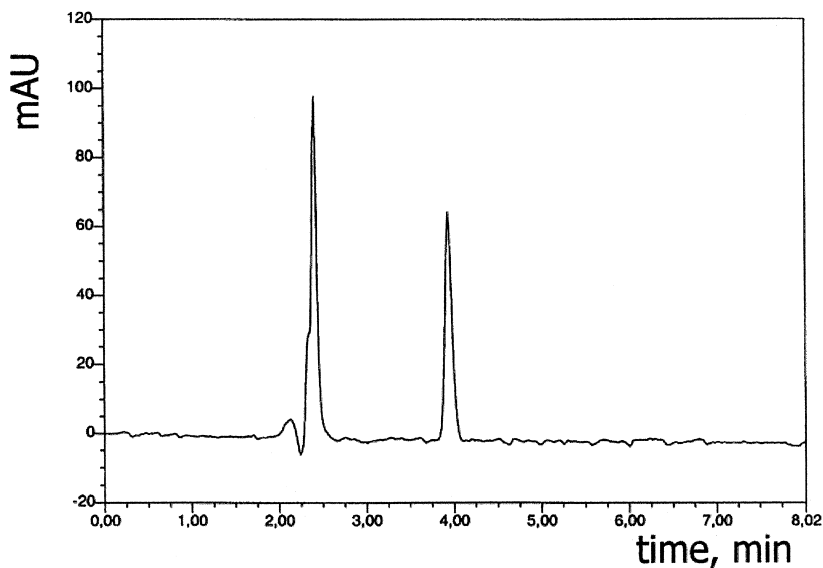


Fig. 13. Separation of α- and β-endosulfan on native titania. Column: 100 Å, 5 μm, 250×4 mm. Eluent: 2-propanol–methanol–*n*-heptane (1:20:480, w/w/w). Flow: 1 cm³/min. Temperature: ambient. Detection: UV at 250 nm.

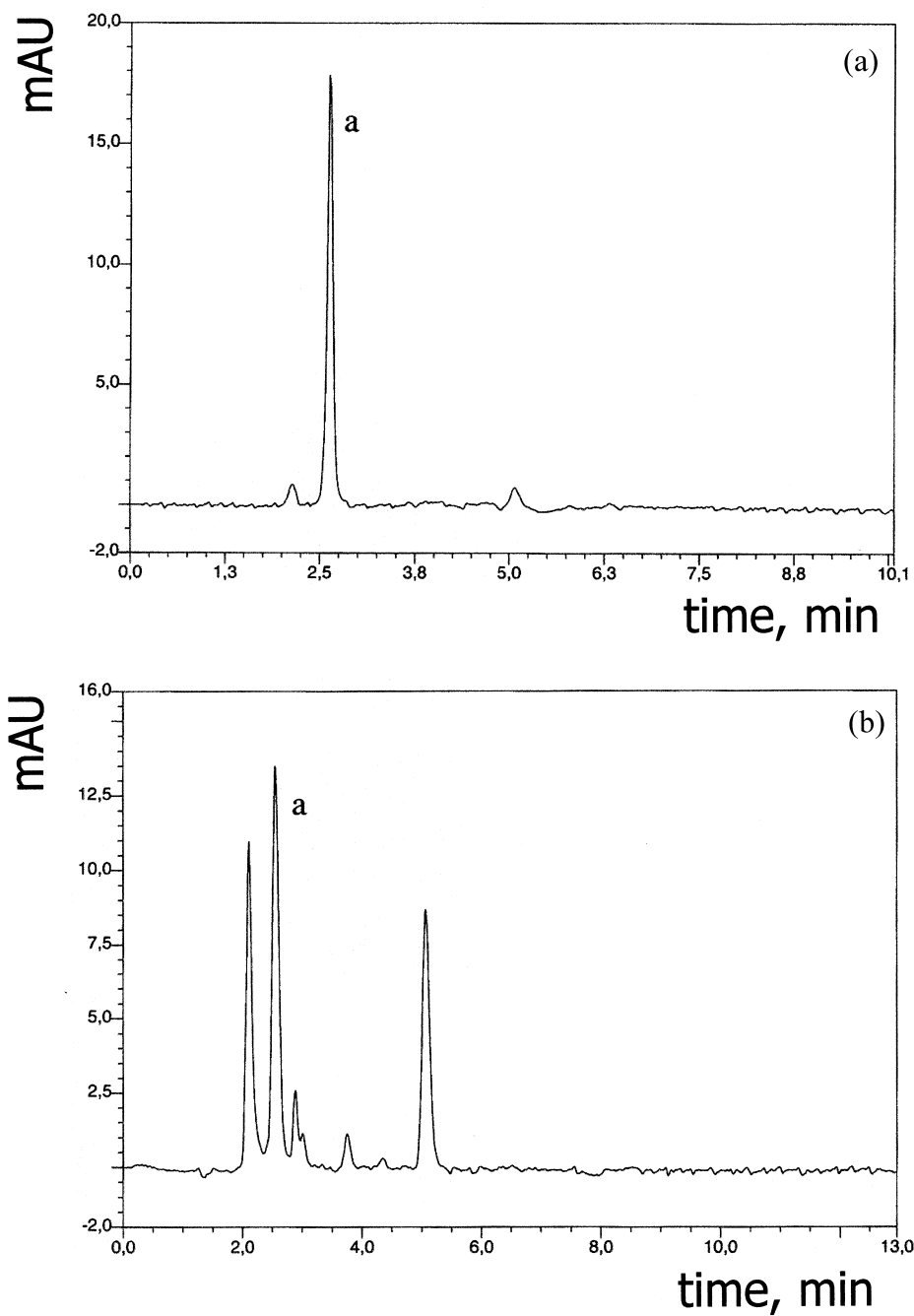


Fig. 14. Chromatograms of fresh (a) and aged (b) solutions of glycyrrhetic acid on native titania. Column: 100 Å, 5 μm, 250×4 mm. Eluent: ethanol–ammonia (25%)–dichloromethane (2:0.08:98, w/w/w). Flow: 1 cm³/min. Temperature: ambient. Detection: UV at 250 nm. Peak a=glycyrrhetic acid.

deterioration products appear at longer and one at a shorter retention time.

4. Conclusions

Sachtopore for HPLC is a crystalline sorbent with either anatase or rutile structure (in the case of 2000 Å wide pore material). It is completely stable against acids as well as bases, thus enabling separations under conditions not accessible using traditional sorbents.

As a crystalline material, it has a lower specific surface area than amorphous sorbents like silica or partly crystalline sorbents like alumina, yet this surface appears to be very homogeneous. Due to this high surface homogeneity titania has a high selectivity for a large variety of different sorbates of interest.

The isoelectric point of this titania sorbent lies between pH 5.5 and 6.5. Its surface hydroxyl groups are therefore only slightly acidic. This enables the use of normal-phase titania to separate basic molecules such as amines, for which otherwise reversed-phase sorbents would be necessary.

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