# Double-layered composite particles as a complex stationary phase for high-performance liquid chromatography

Futaba Honda[a],*, Hirotaka Honda[b], Masumi Koishi[b], Takashi Matsuno[c]

[a]*PHD Inc., 2-49-24, Nakamichi, Hakodate-shi, Hokkaido 041, Japan*
[b]*Faculty of Industrial Science and Technology, Science University of Tokyo, 102-1, Tomino, Oshamambe-cho, Yamakoshi-gun, Hokkaido 049-35, Japan*
[c]*Kitasato University School of Medicine, Kitasato, Sagamihara, Kanagawa 228, Japan*

## Abstract

A composite particle with a double-coating layer consisting of silica (0.3 μm), titania (0.017 μm) and polyethylene beads (13.1 μm) was prepared by a dry impact blending method. The composite particle was employed as a complex stationary phase for high-performance liquid chromatography. Forming the surface double layer caused a change in the surface acidity of the oxides; as a result, the complex stationary phase enhanced the chromatographic retention behavior depending on the ionic interaction. Therefore, the complex stationary phase demonstrates the properties of a novel stationary phase rather than those of a mixture of two stationary phases. By using the multiple retention mechanisms, the separation of some basic drugs and the simultaneous analysis of acids and bases were performed.   © 1997 Elsevier Science B.V.

## 1. Introduction

A high-performance liquid chromatography packing is typical suited to apply surface modification, because it has to simultaneously provide high quality surface chemistry, homogeneity, and mechanical strength [1]. For most packing materials, silica gel or porous polymers are used as the base supports, and then functional groups, such as alkyl groups and ion-exchange groups, are used as the stationary phases by modifying the base supports. The functional groups are usually fixed on the rigid base supports by chemical bonding. Generally speaking,

the packings are powders that have chemically modified surfaces. Thus surface modification plays an important role in improving the properties of powder particles.

We have used a dry impact blending method to prepare the column packings [2,3]; this method is one of the surface modification techniques utilizing physicochemical mechanisms [4–6]. In the field of powder technology the physicochemical forces are defined as a group of adhesion forces between particles. They are especially important for a granulation process utilizing mechanical forces [7,8]. Since the dry impact blending method utilizes such physicochemical forces to modify the particle surfaces instead of simple chemical reactions, the

*Corresponding author.

method can be applied to many kinds of powders independent of their surface properties. By using the dry method, we have obtained the composite particles by individually controlling their shape, hardness, size and surface activity. Furthermore, the composite particles that have surfaces consisting of many functional materials of various ratios can be prepared. They may simultaneously exhibit multiple functions.

This paper describes the preparation, surface characterization and retention behavior for column packings of composite particles with a double-layered stationary phase prepared by the dry impact blending method. The modified particles were made from two kinds of small, hard coating particles as the stationary phases and from large polymer particles as the core particles using a two-step treatment. Silica and titania were selected as the coating particles, because their usefulness for stationary phases and retention characteristics are individually well known (characteristics of titania have been reported in Refs. [9–14]). The experimental focus is how the layered inorganic particles function as the stationary phases during chromatography. The surface properties and the retention characteristics for column packings of the double-layered composite particles were investigated by comparing the single-layered composite particles and a conventional silica particle packing.

## 2. Experimental

### 2.1. Chemicals and reagents

Low-density spherical polyethylene (PE) beads (13.1 μm average diameter, non-porous) as core particles of the composite were supplied by Sumitomo Seika Chemicals (Osaka, Japan). Silica ultramicro-spheres (0.3 μm average diameter, 6 $m^2/$ g specific surface area, non-porous) supplied by Mitsubishi Chemical (Tokyo, Japan) and ultrafine particles of titania (0.017 μm average diameter, 130 $m^2/g$ specific surface area, non-porous, amorphous) supplied by Idemitsu Kohsan (Tokyo, Japan) were employed as the coating particles. The particle size distribution of PE is shown in Fig. 1. The final sizes of every composite particle are dependent on and determined by the PE sizes of the core particles.
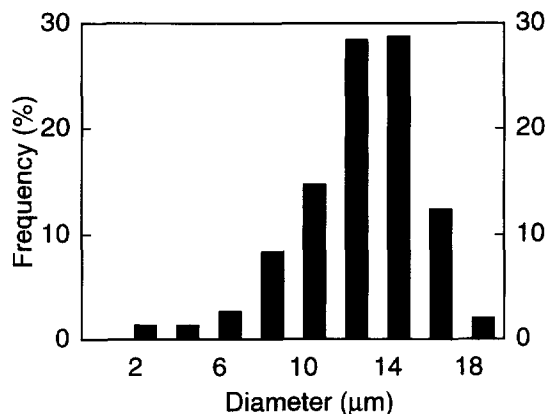


Fig. 1. Particle size distribution of PE beads.

Acetonitrile and other reagents of analytical-reagent grade were purchased from Wako (Osaka, Japan). Quetol-812 (Nisshin EM, Tokyo, Japan) was used as the epoxy resin for embedding the composite particles in order to observe their cross sections. For investigation of the acid–base properties of particles in acetonitrile, methyl yellow, methyl red, neutral red, bromothymol blue, and phenolphthalein, purchased from Wako, and α-naphthyl red hydrochloride, purchased from Aldrich (WI, USA), were employed as indicators. Empty columns (250 mm× 4.0 mm and 150 mm×4.0 mm) were purchased from GL Science (Tokyo, Japan).

### 2.2. Apparatus

For the preparation of composite particles, an O.M. Dizer and a Hybridizer (NHS-0; Nara Machinery, Tokyo, Japan) were used as the machines for combining diverse powders by a dry impact blending method.

Specific surface areas were measured by the BET method with Flowsorb II 2300 (Micromeritics/ Shimadzu, Kyoto, Japan). The surfaces of the composite particles were observed using scanning electron microscopy (SEM) (JSM-T220; JEOL, Tokyo, Japan). Observations of the cross sections were carried out using a field emission scanning electron microscope (JSM-6301F, JEOL). Characterization of the constituent elements of the cross sections was carried out using an electron dispersive X-ray analyzer (EDX) (JED-211, JEOL) attached to

a scanning electron microscope (JSM-5400LV, JEOL).

For investigating the acid–base properties in an aqueous solution, a pH meter (DM-1D; Tokyo Maekawa Chemical, Tokyo, Japan) with a pH electrode (S815 model of measurement, complex type with a sleeve type liquid junction) was employed.

All chromatographic tests were performed on an LC-6A gradient system with a Rheodyne 7125 injection valve, connected to an SPD-6A UV spectrometric detector and a Chromatopac C-R6A (Shimadzu, Kyoto, Japan).

### 2.3. Preparation of composite particles

Details of the equipment and the methods are described below.

In this method, powder particles are dispersed in a high speed air stream, and they frequently and violently collide with each other while spinning. As a result, particle shapes become spherical by compression and by rounding the edges. Furthermore, smaller constituents are fixed onto larger constituent surfaces if powder mixtures of different sizes are treated. The fixation of smaller constituents results from the softening and melting of the surface of either or both constituents due to violent collisions and friction. Some organic and inorganic chemicals melt or become amorphous at temperatures lower than their usual melting points or phase transition points because of the mechano-chemical reaction caused by the physical force employed.

Silica-composite particles (S/P) were prepared as follows. PE (8.5 g) and silica (1.5 g) were blended (1400 rpm, 10 min) with the O.M. Dizer. The resulting mixture was treated by the dry impact blending method using the Hybridizer, with a rotational speed of 16 000 rpm and a treatment time of 10 min. The vessel was cooled by circulation of water through the jacket during every treatment.

Titania-composite particles (T/P) were prepared as follows. PE (9.7 g) and titania (0.1 g) were blended (1400 rpm, 10 min) and treated by the dry impact blending method (10 000 rpm, 3 min). After the addition of further titania (0.1 g), a similar blending and treatment was repeated two more times.

Double-layered composite particles (S/T/P) were prepared in a manner similar to S/P. T/P (8.5 g) and silica (1.5 g) were blended (1400 rpm, 10 min) and treated by the dry impact blending method (16 000 rpm, 10 min).

Each of the composite particles was coated with gold and observed by SEM.

### 2.4. Characterization of the composite particles

The inorganic oxide contents of composite particles were measured as follows. Each of the composite particles (500 mg) was suspended in a mixed solution of xylene and carbon tetrachloride (85:15, 5 ml). After sufficient mixing, the suspensions were heated on a boiling water bath for half an hour, and were centrifuged. The supernatant solutions containing melted polyethylene were removed and precipitations were washed with the mixed solution five times in a similar manner. After drying at 150°C, precipitations were weighed.

### 2.5. Elemental characterization of the composite particles

The cross sections of the composite particles were observed as follows. After the composite particles were embedded in quetol-812 and hardened, the resin blocks were cut with a glass knife attached to a microtome. The cross sections were then coated with carbon. The reflection electron images were observed using field emission SEM. The constituent elements of the cross sections were characterized with the EDX.

### 2.6. Chromatographic tests

Each of the composite particles was slurry-packed into stainless-steel columns attached to a 40-ml packer with n-hexane containing 1.5% isopropanol. The upward or the magnetically stirred slurry techniques were not employed since the composite particles naturally and slowly precipitate in a solvent because of their low densities. After packing, the solvent was replaced with ethanol and acetonitrile in succession. The packer was then removed. All chromatographic experiments were performed at room temperature. Acetonitrile or aqueous acetonitrile was used as the mobile phase. The flow-rate was

0.5 ml/min. Sodium nitrite and methanol were used for measurement of the void volume of the columns.

## 2.7. Acid–base properties of the particle surfaces

The surface acid–base properties of inorganic oxides and composite particles in acetonitrile were measured by the indicator adsorption method [15]. The surface acid–base properties of particles in an aqueous solution were measured by an acid–base titration method [16,17].

For the indicator adsorption method, titania (50 mg), silica (100 mg), and each of the composite particles (100 mg) were suspended in acetonitrile (3 ml), then each 0.1% indicator solution in acetonitrile was added to each suspension. After sufficient mixing, the suspensions were allowed to stand, and coloration of the precipitates was visually observed.

The acid–base titration method was carried out as follows. Silica, titania, and composite particles were previously washed with $n$-hexane, ethanol, acetonitrile, and water successively, followed by vacuum drying. For exclusion of carbon dioxide, all solutions were made from water that was refluxed for 30 min and cooled in a flask attached to a tube filled with sodium hydroxide. Washed silica (300 mg), titania (100 mg), and each of the composite particles (600 mg) were suspended overnight in 2 m$M$ HCl containing 10 m$M$ KCl (100 ml). The pH was recorded for each suspension as it titrated with 0.1 $M$ KOH under a stream of nitrogen to avoid contamination by carbon dioxide. During measurements, a heat insulator was put between a flask containing a suspension and a stirrer to avoid conducting heat from the stirrer. A 10–100 μl dose of the titrant, depending on the solution pH, was added in a successive manner at 1–15 min intervals, since in some cases the solution pH showed a decrease with time. The following titrant was not added until the suspensions maintained a constant pH for at least 30 s. A blank titration curve was also measured used the suspension medium in a similar manner.

Acid and base amounts adsorbed on or released from the particle surfaces were estimated from the difference between each particle's titration curve and the blank titration curve as a function of pH. The surface excess charges per unit mass ($\Delta\Gamma$) on the

particles at each pH and the point of zero charge (pzc) were then calculated.

## 3. Results and discussion

### 3.1. Characterization of composite particles

The inorganic oxide contents of composite particles are 13.7, 3.3, and 17.9% (w/w) for S/P, T/P, and S/T/P, respectively. Nearly every composite particle contains inorganic oxides as they are blended.

Specific surface areas were 1.0, 0.5 and 1.0 m$^2$/g for S/P, T/P, and S/T/P, respectively. Because these values were near the lower limit of values that can be measured by the BET method, a detailed consideration must be avoided. However, since the simple addition of specific surface areas of constituents are ca. 1.5, 4.5, and 4.8 m$^2$/g for S/P, T/P, and S/T/P, respectively, a large amount of the titania surface seems to be lost due to embedding in polyethylene.

### 3.2. Observation of composite particles

SEM photographs of the composite particles are shown in Fig. 2. For the single-layered composite particles, S/P has the surface covered with dense arrangement of silica particles. It has been previously confirmed for S/P that silica particles are tightly fixed on the PE surfaces by being embedded to a depth of ca. 1/10 of their diameter [2]. Silica particles are densely arranged and fixed in contact with each other, so that S/P has a structure similar to a superficially porous particle. On the other hand, T/P has a surface completely covered with the superfine particles.

For the double-layered composite particles (S/T/P), the dense arrangement of silica particles is formed on the T/P surfaces similar to S/P though the surface slightly undulates.

The cross sections of the composite particles were observed using the SEM and analyzed by the EDX for investigating in detail the coating layers. Fig. 3 shows the reflection electron images of the cross sections of the composite particles.
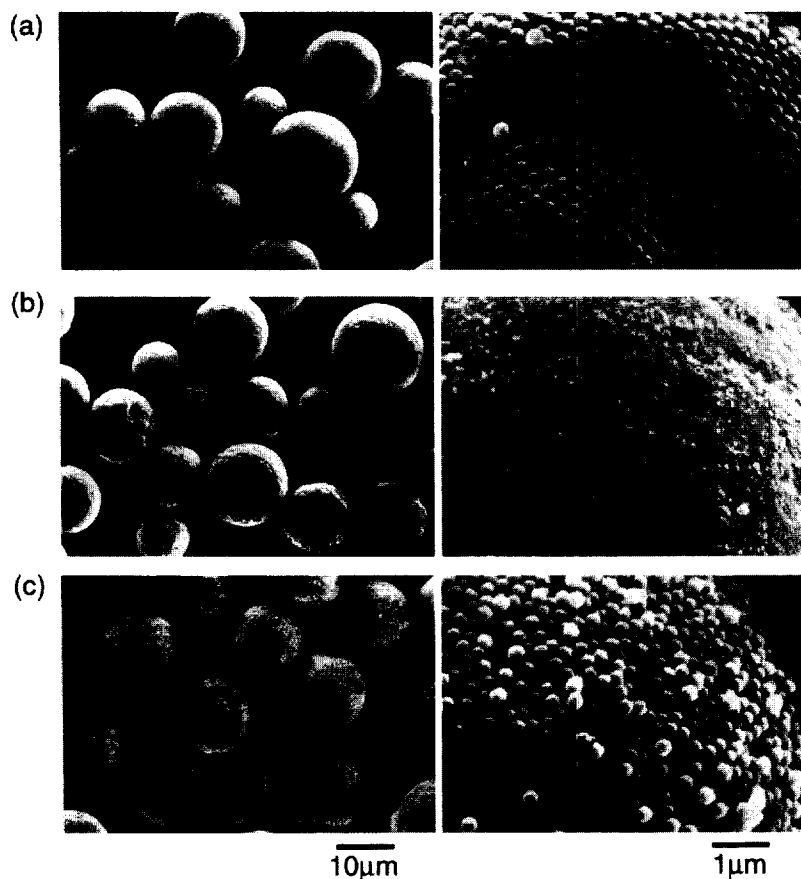
The regular monoparticle arrangement of silica is

Fig. 2. Typical SEM photographs of surfaces of composite particles: (a) S/P; (b) T/P; (c) S/T/P.

observed on S/P (Fig. 3a) and S/T/P (Fig. 3c). In Fig. 3b, though a homogeneous titania layer is present, a thin shell of titania is formed on T/P, the thickness larger than expected from the particle size. Forming the approximately 260 nm thick layer is due to titania being embedded at a variety of depths. It is postulated that the final titania embedded layer thickness corresponds to the thickness of the softening or melting polyethylene layer caused by the impact treatment. For S/T/P, the regular monoparticle arrangement of silica on the titania layers is observed (Fig. 3c).

Furthermore, both Ti and Si were detected on the surface of S/T/P by EDX (Fig. 4). Consequently, it is confirmed that S/T/P has both silica and titania simultaneously on its surface as depicted in Fig. 5.

Using fine particles as coating materials is the unique point of these composite particles. Each coating particle is fixed on core particle surfaces by utilizing the softening or melting property, for a given treatment condition of the core particles as an adhesional binder. The process enables the core particles to have a double-coating layer irrespective to the surface properties of each wall particle. Furthermore, the method enables the core particles to form different nano- or micrometer-level fine structures on their surfaces. In this case, the arrangement of silica particles forms a regular superficially fine porous microstructure on the PE particle surfaces, and the titania particles are regarded just like an inner precoated layer of porous S/P particles, as depicted in Fig. 5.
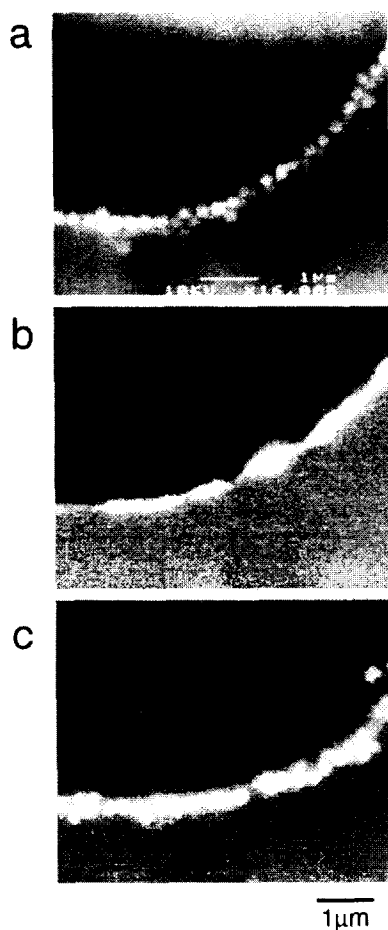
1μm

Fig. 3. Typical SEM photographs of cross sections of composite particles: (a) S/P; (b) T/P; (c) S/T/P.

## 3.3. Retention properties for column packings

We considered the S/T/P surface as a layered-stationary phase, and its retention behavior was studied. S/P, T/P and S/T/P were each packed into stainless columns, then some organic compounds were analyzed. The organic compounds were classified into three categories based on the main interaction with the stationary phases. The capacity factors ($k'$) on each column are shown in Table 1.

The retention behaviors for the polycyclic aromatics and substituted aromatics were nearly equal on every column. However, T/P and S/T/P, which contain the titania layer, differed in $k'$ of the polar compounds from S/P. S/T/P significantly retained compounds having not only a carboxyl group but also a phenol group similar to T/P. On the other hand, the $k'$ values for basic compounds on S/T/P were lower than that on T/P. These results suggest that the titania layer adds to the retention of polar solutes. Also there are two explanations for the retention behavior of the basic solutes. One is that silica, being fixed so close to titania, affects the surface polarity of titania. Another is that the silica layer prevents solute access to the titania.

## 3.4. Acid–base properties of the particle surfaces in acetonitrile

The surface charge of each composite particle seemed to influence their retention behavior, because they exhibited unique properties for polar solutes. Silica and titania have surface hydroxy groups that play an important role in the retention by forming hydrogen bonds with solute molecules during normal-phase chromatography.

First, the surface acidities in acetonitrile were examined using the indicator adsorption method. In this method, acid or base intensity of a solid surface is judged by the degree of surface adsorption of each indicator of different $pK_a$. The indicators' $pK_a$ (or $pK_b$) and the observation results are shown in Table 2.

It is confirmed that silica, S/P, and S/T/P have acidic sites of $4.8 < pK_a < 6.8$ because they were colored only by neutral red. On the other hand, titania and T/P have acidic sites of $4.0 < pK_a < 4.8$ because they were colored by neutral red and methyl red. These results mean that the surface hydroxy groups on titania possess stronger acidity than on silica in acetonitrile. The high acidic sites tend to release protons and to attract acidic groups. It seems to contribute to the extremely high retention of polar solutes shown in Table 1.

S/T/P did not show the same strong acidity as T/P, although it retained acidic solutes similar to T/P. It is thought that the outer silica layer concealed the colored titania layer from view. At the same time, this indicates that the acidity of the outer silica surface did not shift very much from independent silica.
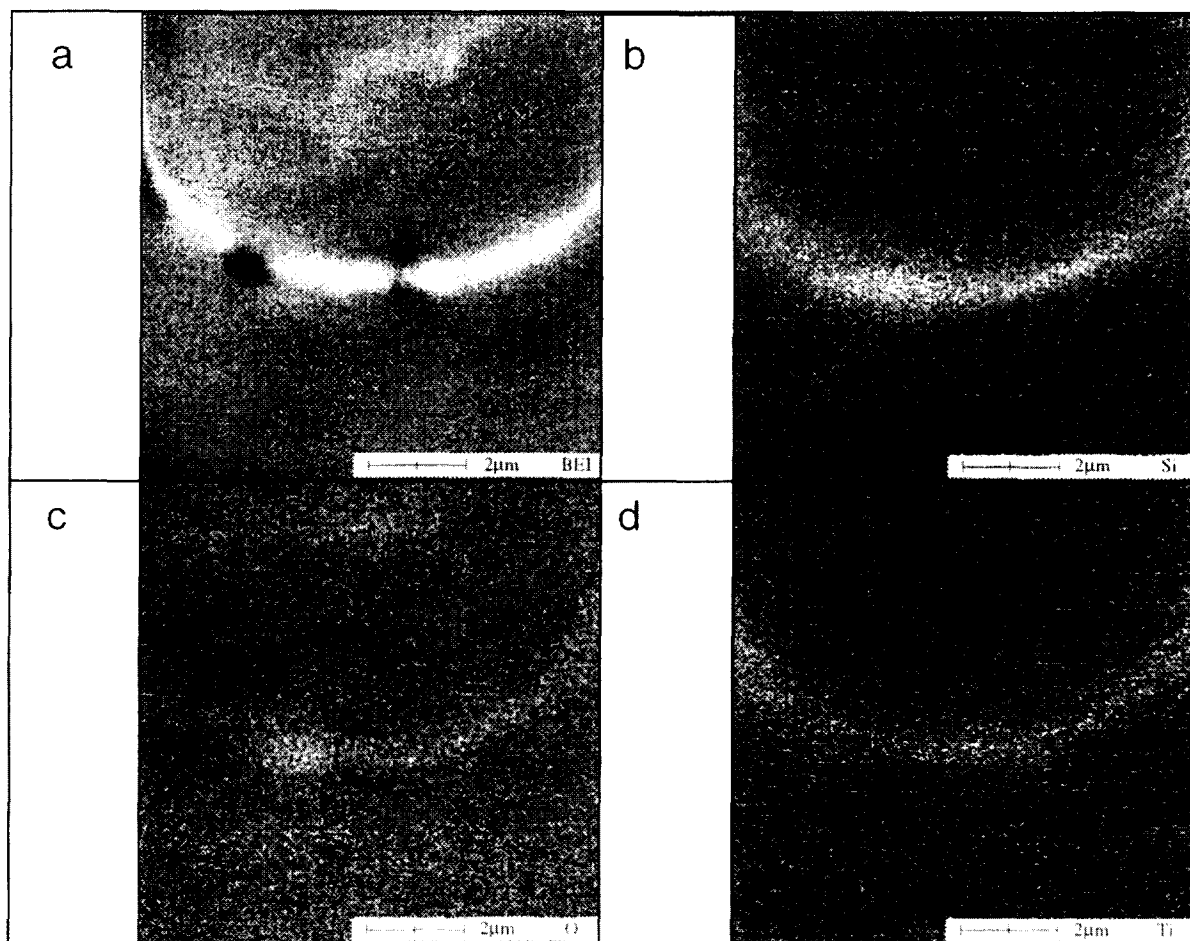
Fig. 4. Elemental characterization of the cross section of S/T/P. (a) secondary image of the cross section; (b) distribution of Si; (c) distribution of O; (d) distribution of Ti.
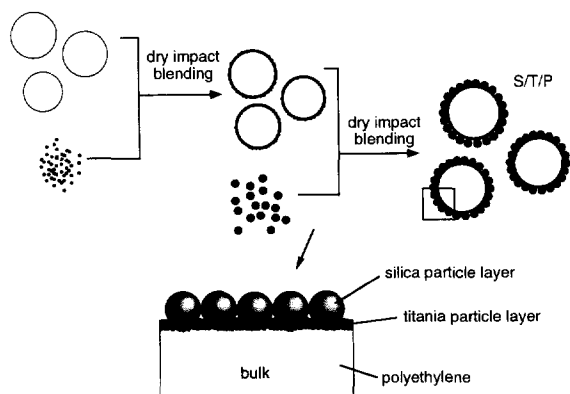


Fig. 5. Schematic diagram of S/T/P.

## 3.5. Acid–base properties of the particle surfaces in an aqueous solution

Next, the surface properties of composite particles in an aqueous solution were investigated using the titration method. The titration method, which was developed to investigate the surface evaluation of catalysts, is a very accurate method since it can count such weak acid sites that can not be counted by the gas adsorption method which is one of the typical estimating methods.

Titration curves of the S/T/P suspension and of the blank solution are shown in Fig. 6. The difference in KOH solution consumption between the

Table 1
k' values on the composite particle packed columns with acetonitrile as the mobile phase

| Solutes | S/P | T/P | S/T/P |
|---|---|---|---|
| *Effect of π–π interactions* | | | |
| Benzene | 0.19 | 0.14 | 0.14 |
| Fluorene | 0.20 | 0.14 | 0.15 |
| Anthracene | 0.31 | 0.22 | 0.23 |
| | | | |
| *Effect of substitutes* | | | |
| Methyl benzoate | 0.04 | 0.02 | 0.03 |
| Butyl benzoate | 0.06 | 0.04 | 0.05 |
| Isoamyl benzoate | 0.06 | 0.03 | 0.03 |
| Nitrobenzene | 0.02 | 0.01 | 0.01 |
| Toluene | 0.25 | 0.18 | 0.19 |
| | | | |
| *Effect of hydrogen bonds* | | | |
| Phenol | 0.01 | >20 | >20 |
| Benzoic acid | 2.10 | >20 | >20 |
| Aniline | 0.01 | 0.18 | 0.16 |
| Pyridine | 0.09 | 6.77 | 2.72 |
| Nicotinic acid | >20 | >20 | >20 |



Fig. 6. Titration curves of S/T/P suspension and a blank solution. ○, blank solution; ●, S/T/P suspension.

suspension and the blank at each pH gives the acid or the base amount adsorbed on or released from the S/T/P surface. The surface excess charge per unit mass ($\Delta\Gamma$) was calculated from the difference in the titration curves. Although a result of one measurement for each sample is indicated in Figs. 7–10, the pzc (the pH at which both excess charges are just equal, that is to say, the point of zero charge) obtained from three measurements were within pH±0.15 for S/T/P.

The results of silica and titania are shown in Fig. 7. Silica and titania exhibited both a negative and positive excess charge in the measured range. The pzc is 6.8 and 4.3 for the silica and titania, respec-

tively. These results agree with the acidity in acetonitrile. The $\Delta\Gamma$ curves of the oxide physical mixture that contained silica and titania at the same ratio to S/T/P are also shown in Fig. 8. As shown in Fig. 8, the mixture's $\Delta\Gamma$ curves almost agreed with the calculated curves evaluated by proportionally adding $\Delta\Gamma$ values of silica and titania in Fig. 7. This means that the additivity of $\Delta\Gamma$ holds for the physical mixture of oxides.
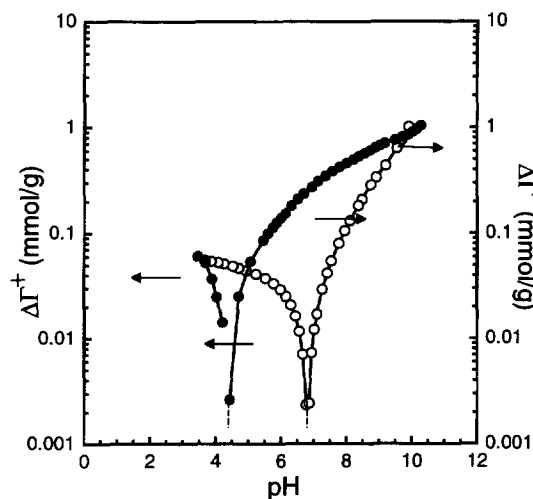
The $\Delta\Gamma$ curves of the composite particles are

Table 2
The acid–base property of the particles by an indicator adsorption method

| Indicators | $pK_a$ | Silica | Titania | S/P | T/P | S/T/P |
|---|---|---|---|---|---|---|
| Dimethyl yellow | 3.3 | – | – | – | – | – |
| α-Naphthyl red | 4.0 | – | – | – | – | – |
| Methyl red | 4.8 | – | + | – | + | – |
| Neutral red | 6.8 | + | + | + | + | + |
| Bromothymol blue | 7.2[a] | – | – | – | – | – |
| Phenolphthalein | 9.3[a] | – | – | – | – | – |

–: not colored, +: colored.
[a] $pK_b$.



Fig. 7. Log $\Delta\Gamma^+$ and log $\Delta\Gamma^-$ against pH plots for silica and titania. ○, silica; ●, titania; - - -, pzc.
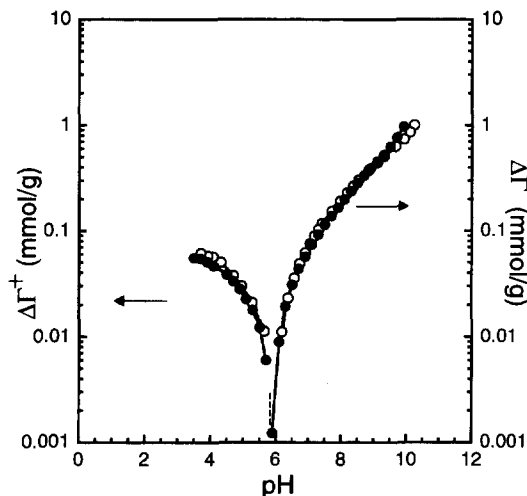
Fig. 8. Log $\Delta\Gamma^+$ and log $\Delta\Gamma^-$ against pH plots for the mixture containing silica and titania. ○, measured; ●, calculated from silica and titania; - - -, pzc.



Fig. 10. Log $\Delta\Gamma^+$ and log $\Delta\Gamma^-$ against pH plots for S/T/P. ○, measured; ●, calculated from S/P and T/P; - - -, pzc.

shown in Fig. 9. The pzc of S/P, T/P and S/T/P was 7.1–7.3, <2.7 and 6.8–7.0, respectively. S/P showed a higher pzc than silica by ca. pH 0.4. In contrast, T/P possesses the negative excess charge at the whole area measured. As a result, the pzc was not detected even at pH 2.7, then the pzc was lower than that of titania by not less than pH 1.6. These results suggest that combining oxides with PE
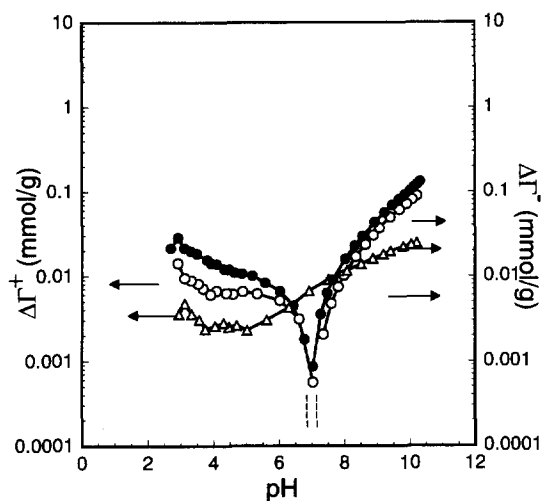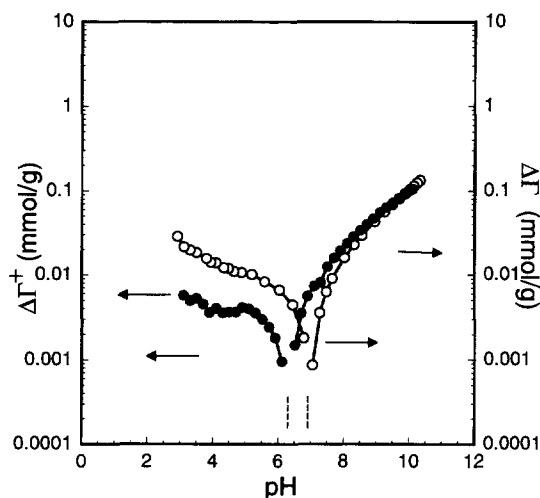


Fig. 9. Log $\Delta\Gamma^+$ and log $\Delta\Gamma^-$ against pH plots for S/P, T/P, and S/T/P. ○, S/P; △, T/P; ●, S/T/P; - - -, pzc.

changes the acid–base properties of the oxide surfaces.

Fig. 10 shows the comparison of the measured and calculated $\Delta\Gamma$ curves of S/T/P. The calculated curves were evaluated by adding the $\Delta\Gamma$ of S/P and T/P. The measured pzc is higher than the calculated pzc by ca. pH 0.6, and higher than the calculated pzc from the simple inorganic oxides (see Fig. 8) by ca. pH 1.1. Therefore, S/T/P showed an acid–base property different from not only the mixture of titania and silica but also the mixture of the single layered composite particles. Furthermore, the measured $\Delta\Gamma^+$ values were over fivefold higher than the calculated $\Delta\Gamma^+$ values at pH 3–6.

Consequently, the acid–base property of S/T/P was different in not only quality but also quantity from that simply estimated. Such a shift of pzc and the increase in $\Delta\Gamma$ were never observed for the physical mixture of oxides. These phenomena may be interpreted by the increase in exposed titania surface area at S/T/P. There is a possibility of increase in surface area due to the exposure of a fresh surface upon collision during the second coating process. However, since the pzc of titania is pH 4.3, it seems to be inadequate that the increase in the titania surface contributes to the shift of pzc to higher pH and the increase in $\Delta\Gamma^+$ at pH 3–6. Therefore, the generation of an electrical interaction

between oxides and also between oxides and PE is suggested.

### 3.6. Properties for column packings with aqueous mobile phases

Since three kinds of composite particles differed in surface chemistry, retention behavior for some polar compounds was studied with aqueous mobile phases. Capacity factors obtained using the mobile phase of 97% and 10% acetonitrile in water are indicated in Table 3.

As compared with Table 1, phenol, pyridine, and aniline decrease in $k'$ values on T/P and S/T/P significantly by the addition of 3% water. However, T/P and S/T/P exhibited a strong retention for benzoic acid with 97% acetonitrile. The specific high retention for carboxyl groups was also pointed out in papers that dealt with the use of sintered titania (rutile form) [9–14] and zirconia [9,10,13,14,18–24] for column packings. The retention behavior was attributed to the Lewis acid sites on them [13,19–24].

In contrast, S/P retained basic solutes more strongly than did T/P, when 10% acetonitrile was used as the mobile phase. Here, S/T/P exhibited higher $k'$ values both of benzoic acid at 97% acetonitrile and basic solutes at 10% acetonitrile than T/P and S/P. These results indicate that S/T/P maintains the chromatographic characteristics of each silica and titania for low- and high-water-content conditions. Moreover, it retains solutes more strongly than both single materials for each condition.

To clarify the retention mechanisms of the composite particles for each condition, the retention be-

havior of the polar solutes was investigated under some conditions.

First, for investigating the effect of water on retention of polar solutes, the correlation between water content of the mobile phase and $k'$ of benzoic acid at a low water content was measured. In Fig. 11, S/T/P and T/P decreased $k'$ according to the increase in water content. Since they decreased steeply by the slight increasing in water content, the water adsorbed stationary phase seems to retain hardly benzoic acid. Especially, S/T/P remarkably decreased $k'$ even with the small addition of water. Both $k'$ values of S/T/P and T/P are almost similar at 95% acetonitrile though the $k'$ on S/T/P is 1.8 times as large as the $k'$ on T/P at 97% acetonitrile. However, since S/P hardly retained benzoic acid under these conditions, the higher $k'$ on S/T/P seems not to be due to the simple addition of retention by silica and titania.

Next, the retention behavior of polar solutes under aqueous conditions was investigated. Under aqueous conditions, the ionic interaction between the hydroxyl groups on stationary phases and solutes is thought to be a main retention mechanism. Every mobile phase contains 10% acetonitrile to avoid solute adsorption by hydrophobic interaction because a certain amount of PE is exposed on the composite particles. The concentration and pH of the mobile phases denote those of the aqueous parts before mixing with acetonitrile.

Table 3
$k'$ of the composite particles packed columns with aqueous acetonitrile as the mobile phase

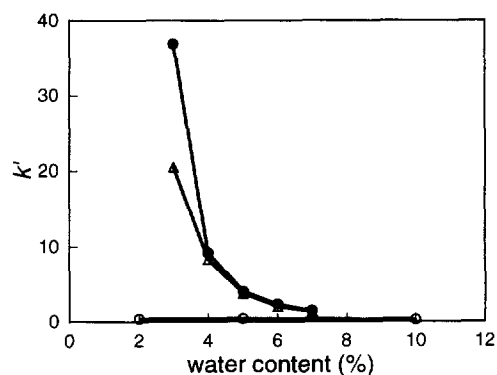| | 97% Acetonitrile | | | 10% Acetonitrile | | |
|---|---|---|---|---|---|---|
| | S/P | T/P | S/T/P | S/P | T/P | S/T/P |
| Phenol | 0.07 | 0.01 | −0.03 | 0.04 | 0.29 | 0.22 |
| Benzoic acid | 0.13 | 20.52 | 36.93 | 0.02 | 0.73 | 0.97 |
| Aniline | 0.08 | −0.03 | −0.02 | 0.42 | 0.27 | 0.43 |
| Pyridine | 0.12 | 0.01 | 0.13 | 1.32 | 0.17 | 1.64 |



Fig. 11. The correlation between water content of the mobile phase and $k'$ of benzoic acid. ○, S/P; △, T/P; ●, S/T/P: mobile phase, aqueous acetonitrile; flow-rate 0.5 ml/min; detection 254 nm.
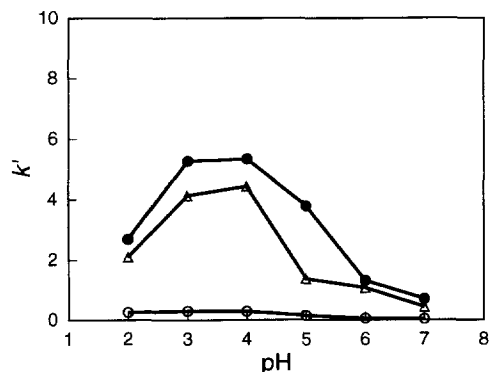
Fig. 12. The correlation between pH of the mobile phase and $k'$ of benzoic acid. $\bigcirc$, S/P; $\triangle$, T/P; $\bullet$, S/T/P: mobile phase, 0.1 $M$ NaClO$_4$ containing 10% of acetonitrile (pH is controlled by HClO$_4$ or NaOH); flow-rate 0.5 ml/min; detection 254 nm.



Fig. 14. The correlation between pH of the mobile phase and $k'$ of pyridine. $\bigcirc$, S/P; $\triangle$, T/P; $\bullet$, S/T/P: mobile phase, 0.1 $M$ NaClO$_4$ containing 10% of acetonitrile (pH is controlled by HClO$_4$ or NaOH); flow-rate 0.5 ml/min; detection 254 nm.

Fig. 12 shows the correlation of the mobile phase pH and $k'$ of benzoic acid. Benzoic acid exhibits a high $k'$ at pH 3–4 especially on T/P and S/T/P. On the other hand, as the NaClO$_4$ concentration in the mobile phase decreased at pH 4, S/T/P increases in $k'$ while T/P scarcely varies in $k'$ (Fig. 13). The results that the NaClO$_4$ concentration scarcely affects $k'$ values of benzoic acid on T/P agree with the reports that Lewis acids adsorb carboxyl groups by the ligand exchange interaction. Therefore, S/T/P retains benzoic acid by utilizing the ligand exchange interaction and the simple ionic exchange interaction simultaneously. Since S/P scarcely demonstrates
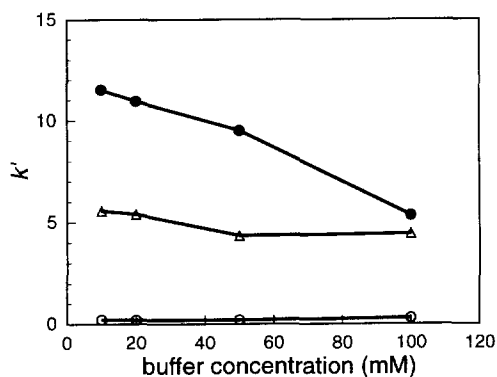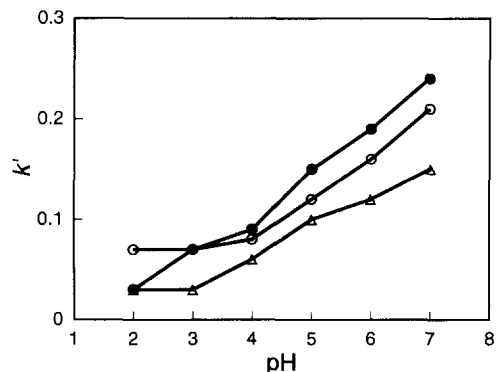
such an ionic exchange behavior in Fig. 13, the simple addition of the properties of silica and titania never causes the interaction observed on S/T/P.

Fig. 14 shows the correlation of $k'$ of pyridine and pH of the mobile phases. Every $k'$ increases uniformly with an increase in pH above pH 3. And the $k'$ values decrease on every column when the NaClO$_4$ concentration in the mobile phase is increased at pH 5 (Fig. 15). Therefore, every composite particle seems to retain pyridine by utilizing the simple ionic exchange interaction. S/T/P exhibits the highest $k'$ at every point on both graphs except at
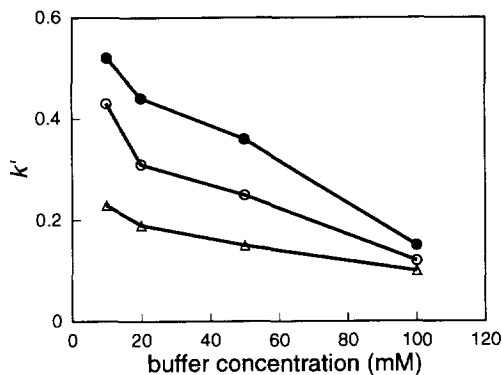


Fig. 13. The correlation between salt concentration of the mobile phase and $k'$ of benzoic acid. $\bigcirc$, S/P; $\triangle$, T/P; $\bullet$, S/T/P: mobile phase, NaClO$_4$–HClO$_4$ (pH 4) containing 10% of acetonitrile; flow-rate 0.5 ml/min; detection 254 nm.



Fig. 15. The correlation between salt concentration of the mobile phase and $k'$ of pyridine. $\bigcirc$, S/P; $\triangle$, T/P; $\bullet$, S/T/P: mobile phase, NaClO$_4$–HClO$_4$ (pH 5) containing 10% of acetonitrile; flow-rate 0.5 ml/min; detection 254 nm.

pH 2 and 3. Although the reason for low $k'$ values at low pHs is not proved, on S/T/P, both silica and titania retain pyridine additively at other points.

Consequently, S/T/P maintains the chromatographic properties derived from silica and titania as shown in the retention behavior under low- and high-water content conditions. Furthermore, S/T/P demonstrates higher retention for benzoic acid than expected from the addition of S/P and T/P. Evidently, it seems that the ionic exchange interaction contributes to such high retention. The ionic exchange ability must be caused by layering the stationary phases; this suggests that some interactions are generated on the S/T/P surface between the extremely closed surfaces of both inorganic particles.

We assumed that static electrification contributes to the generation of such ability. Some materials generate static electrification on their surfaces by contacting with other materials because of exchanging electrons across the contact surface [25,26]. On the surface of S/T/P, the hydroxy groups that differ in polarity on them contact each other. The surface electrification and the electrical interaction between these hydroxy groups are considered to contribute to the shift and the enhancement in the charges.

### 3.7. Utilization as a packing for separation of basic drugs

From the examination of chromatographic characteristics, S/T/P exhibits some retention mechanisms. The coexistence of such multiple functions as stationary phases on one particle provides the unique properties of S/T/P. We then applied S/T/P to the analysis of some compounds by utilizing these fundamental properties. Basic and acidic drugs were selected as solutes, because S/T/P resists mobile phases containing an organic solvent and simultaneously retains both acidic and basic groups.

Chromatograms of basic drugs on the S/T/P column and a conventional silica column are shown in Fig. 16. On S/T/P, according to the increase in pH, the retention time of propranolol and chlorpheniramine are extended. These drugs are easily separated above pH 3. On the other hand, a high pH condition, above pH 5, was required for the separation of these drugs on the silica column. Proprano-
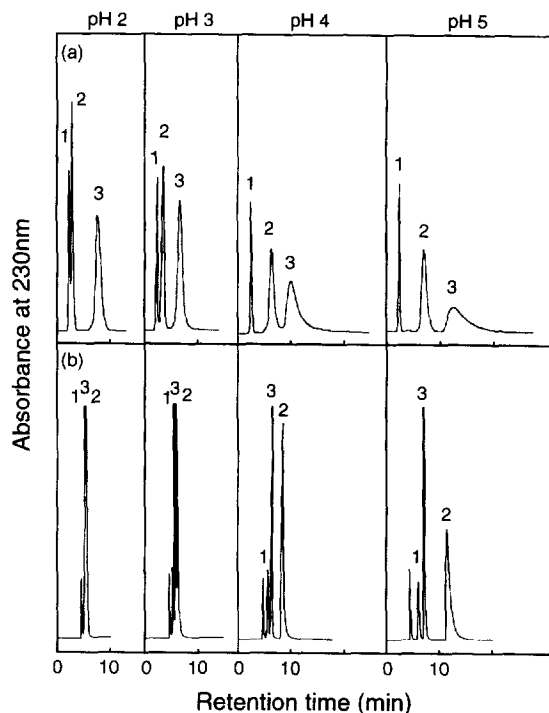


Fig. 16. Chromatograms of basic drugs on the S/T/P column and a conventional silica column. (a) S/T/P; (b) CLC-SIL; mobile phase, pH-controlled 0.1 $M$ NaClO$_4$-HClO$_4$ containing 15% of acetonitrile; flow-rate 0.5 ml/min; detection, 230 nm; peaks: 1, procaine; 2, chlorpheniramine; 3, propranolol.

lol was the last to elute from the S/T/P column, though it was the second to elute from the silica column. These results clearly suggest that S/T/P retains these drugs by a mechanism different from that of the silica column. Since the difference in the mechanism is mainly thought to come from titania, the $k'$ of drugs was measured with the mobile phase containing an acetic buffer, which is a Lewis base (Table 4). Propranolol significantly decreases $k'$ though others showed only a slight decrease. It proves that S/T/P strongly retains propranolol by utilizing a Lewis acid–base interaction. The chemical structure of propranolol suggests that Ti$^+$ on titania interacts with the hydroxy group and the secondary amino group on propranolol. Therefore, S/T/P can more precisely distinguish these drugs by not only amino groups but also by multiple functional groups' surface.

Table 4
Capacity factor of basic drugs obtained by mobile phases with and without Lewis base

|  | Mobile phase[a] | |
|  | 0.1 M NaClO$_4$–HClO$_4$ (pH 3) | 0.1 M sodium acetate buffer (pH 3) |
| --- | --- | --- |
| Procaine | 0.21 | 0.10 |
| Chlorpheniramine | 2.01 | 1.59 |
| Propranolol | 9.29 | 0.58 |

[a] Mobile phases contain 90% of each aqueous solution and 10% acetonitrile.

Finally, to use the advantage of S/T/P, simultaneous analysis of basic and acidic drugs was performed. Fig. 17a,b show the separation of acidic and basic drugs under a linear gradient of aqueous acetonitrile and an aqueous solution. A solute, poorly soluble in water, like indomethacin, can be analyzed by using the ligand exchange interaction under a high concentration of acetonitrile (Fig. 17a). Also, by using both ionic and ligand exchange interactions, four drugs were successfully separated (Fig. 17b). The simultaneous analysis of basic and acidic solutes is usually complicated. Though it can be performed with ion-exchange or paired-ion chromatography by losing the ionicity of either or both solutes, these techniques require some steps for the separation, and

the regulation of analytical conditions is difficult. S/T/P can analyze them by retaining both anions and cations under an appropriate pH as shown in Fig. 17.

Consequently, a stationary phase that simultaneously demonstrates multiple retention mechanisms is useful as packing for analyzing compound mixtures that contain acids, bases, and compounds that have some functional groups.

There are some studies for utilizing multiple materials as stationary phases at the same time. The mixed stationary phases are prepared by surface precipitation [27], or a combination of two kinds of packings [28]. For these studies, the existence ratio of the stationary phases is treated at a macro level,
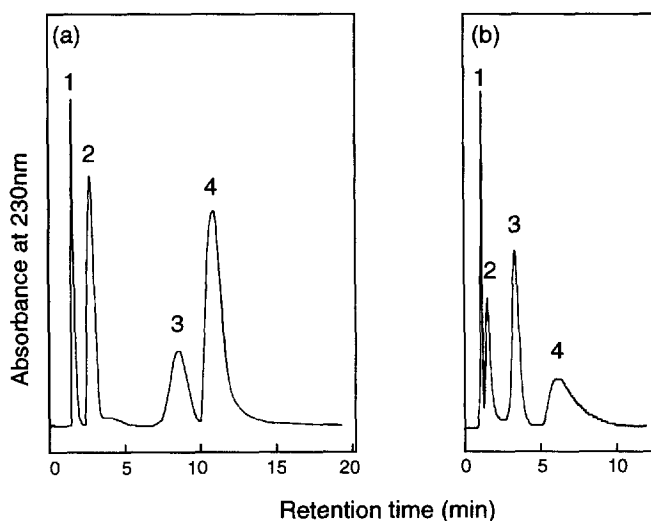


Fig. 17. Separation of basic and acidic drugs on the S/T/P column. (a) Mobile phase, 20-min linear gradient from 95 to 50% acetonitrile; flow-rate, 0.5 ml/min; detection, 230 nm; peaks: 1, phenol; 2, procaine; 3, indomethacin; 4, chlorpheniramine. (b) Mobile phase, 0.1 M NaClO$_4$–HClO$_4$ (pH 5) containing 15% of acetonitrile; flow-rate 0.5 ml/min; detection, 230 nm; peaks: 1, phenol; 2, aspirin; 3, chlorpheniramine; 4, propranolol.

and no attention is paid to the distribution of each stationary phase in the whole packing. Furthermore, each functional group or material works independently as a stationary phase.

At the start of this study, we expected the dry impact blending method to be useful for preparing the stationary phases by preserving the homogeneity of multiple materials on one particle. However, the double-layered composite particle exhibits unique chromatographic properties different from both the single-layered composite particle of silica and titania. This means that a novel complex stationary phase is formed on the double-layered composite particles. Thus the structural factor and size effect of arrangement of fine particles must play an important role in forming the novel stationary phase. S/T/P is a new material with an artificially controlled surface in a sense.

## 4. Conclusion

Composite particles with a double-layer of silica and titania as the stationary phase as prepared in this study is a complex at a fine particle level. The composite particle has been expected to be useful in preserving the homogeneity of the stationary phase when multiple materials are simultaneously used as a stationary phase. However, it is found that the surface acidity of the double-layered composite particles was distinctly changed from the physical mixture of silica and titania. These results suggest that the close fixing of oxides, such as silica and titania, on PE surfaces significantly increases the electrical interaction between both oxide surfaces. From the chromatographic characterization, S/T/P maintains the chromatographic characteristics of both silica and titania, furthermore, it retains polar solutes more strongly than expected from each single-layered composite particle.

Therefore, the double-layered composite particles isn't a mixture of two stationary phases, but is a novel stationary phase that demonstrates retention mechanisms never observed for the single-layered stationary phases. By using the multiple retention mechanisms presented for one packing particle, S/T/P was useful for the separation of some basic drugs and the simultaneous analysis of acids and bases.

From these results, S/T/P is expected to be more useful for analysis of some drugs or vital constituents than conventional packings consisted of single materials. Because these compounds have many analogues, multiple recognition sites on S/T/P are effective for recognizing slight differences in their chemical structures. Furthermore, S/T/P can analyze samples which contain amphoteric, acidic, and basic compounds simultaneously under a simple and a mild condition.

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