Footprint Catalysis. III.[1,2] Inducible Alteration of Substrate Specificities of Silica(Alumina) Gel Catalysts for 2,4-Dinitrophenolysis of Toluic Anhydrides by Footprint Imprinting

Kensaku MORIHARA,* Eiko TANAKA, Yuko TAKEUCHI, Kazue MIYAZAKI, Nami YAMAMOTO, Yoshimi SAGAWA, Eriko KAWAMOTO, and Toyoshi SHIMADA
Department of Chemistry, Faculty of Science, Nara Women's University, Kitauoyanishi-machi, Nara 630
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A tailor-made catalyst design was attempted for a required substrate specificity by the authors' footprint imprinting method. Two silica(alumina) footprint catalysts were prepared by imprinting with *N*-benzoylbenzenesulfonamide (**1**) and *N*-(*p*-toluoyl)-*p*-toluenesulfonamide (**2**) from the same aluminum ion-doped silica gel. Their catalytic effects upon 2,4-dinitrophenolysis of benzoic anhydride, *p*-, *m*-, and *o*-toluic anhydrides were compared in order to find any difference in substrate specificities. The catalyst prepared by imprinting with **1** showed the largest specificity(k_{cat}/K_m) toward benzoic anhydride, whereas the catalyst prepared with **2** showed the largest specificity toward *p*-toluic anhydride. This finding evidently demonstrated that the substrate specificities of footprint catalytic sites are alterable, as required through selecting appropriate transition state analogs as template molecules for footprint marking.

Previous papers[1,2] of this series reported that unusual catalytic sites of Lewis acid could be marked by imprinting on a silica gel surface like the footprints of the template molecules with a complementary structure to them, and that these "footprint" catalytic sites possessed an exact molecular recognition capability toward the template molecules used in the imprinting process, a capability which was revealed as being an abnormally strong competitive inhibition in a catalysis. This molecular recognition of footprint catalytic sites should also be revealed in their substrate specificity, which would lead to a catalyst design for a required substrate specificity in demand.

During the course of previous studies,[1,2] butanolysis[3] of benzoic anhydride using a potentiometric titration[4] was employed as a catalytic activity assay system. However, several kinds of technical problems were encountered in which the butanolyses of benzoic anhydride proceeded very slowly, accompanying the uncatalyzed reaction in significant quantities. Also, potentiometric titration[4] was rather troublesome, lacking sensitivity, and less reliable for an accurate assay. Accordingly, an improved assay system has been necessarily required for the further investigation of substrate specificities upon the footprint catalytic sites.

The present report deals with two matters. The first is with regard to a new photometric assay system for catalytic activity using 2,4-dinitrophenolysis of benzoic anhydride, and its application to a method for catalytic sites titration with the aid of pyridine poisoning of the catalysts. The second regards an attempt to alter the substrate specificities of silica(alumina) catalysts by selecting appropriate transition state analogs for the reaction of the substrate as template molecules. The kinetic parameters in 2,4-dinitrophenolysis of two footprint catalysts and of a control silica(alumina) catalyst were measured upon four anhydride substrates, i.e., benzoic anhydride and *p*-, *m*-, *o*-toluic anhydrides; induced alterations of substrate specificities were clearly demonstrated.

Experimental

Materials. All chemicals were of the guaranteed grade of Nacalai Tesque Co., Ltd., if not specified.

Templates and Inhibitors: *N*-Benzoylbenzenesulfonamide (Ph-SO_2-NH-Bz, **1**) and *N*-(*p*-toluoyl)-*p*-toluenesulfonamide (CH_3-C_6H_4-SO_2-NH-CO-C_6H_4-CH_3, **2**), and dibenzamide (Ph-CO-NH-CO-Ph, **3**) were of the same preparations as those previously reported.[1,2] Pyridine and 2,6-dimethylpyridine were of guaranteed grade reagents and were used without further purification.

Silica Gel: Merck Kieselgel 60, art. no. 7754, particle size 0.06—0.20 mm, mesh 70—230, was used.

Substrate: Benzoic anhydride was recrystallized from benzene-petroleum benzine, mp 42 °C (lit,[5] 42 °C). Three toluic anhydrides were prepared according to a procedure described in the literature.[6] *p*-Toluic anhydride was recrystallized from ethanol, mp 95—95.5 °C (lit,[7] 95 °C). IR (Perkin-Elmer 1740) (KBr) 3050 (arom.), 1775, 1715 (C=O, anhydride), 1609 (arom.), 1449, 1376 (methyl), 840, 824 cm^{-1} (*p*-, arom.). *m*-Toluic anhydride was recrystallized from ether-petroleum ether, mp 67.0—68.5 °C (lit,[8] 71 °C). IR (KBr) 3070, 3036 (arom.), 1784, 1718 (C=O), 1591 (arom.), 1487, 1378 (methyl), 803, 743, 724 cm^{-1} (*m*-, arom.). *o*-Toluic anhydride was recrystallized from ether-petroleum benzine, mp 36.5—38.0 °C (lit,[9] 36—38 °C, 39 °C). IR (KBr) 3074 (arom.), 1784, 1719 (C=O), 1456, 1381 (methyl), 733 cm^{-1} (*o*-, arom.).

Nucleophile: Potassium 2,4-dinitrophenolate was prepared through the neutralization of 2,4-dinitrophenol in a minimum amount of methanol with concd aqueous potassium hydroxide; the salt was recrystallized from hot water, and dried at 130 °C in vacuo.

Solvent: Acetonitrile, previously dehydrated with calcium chloride, was dried over phosphorus pentoxide overnight, and distilled using a Hempel fractionating column, bp 81—82 °C. To the distillate was added calcium hydride and distillation was repeated to remove perfectly any trace of water or acidic impurities.

Catalysts: Three footprint catalysts were prepared according to an imprinting method which was previously described in detail,[1] using **1**, **2**, **3**, as templates, respectively, (they are referred hereafter as catalyst {**1**}, {**2**}, and {**3**} in text.) as follows.

Kieselgel (100 g) was refluxed for 6 h with concd hydrochloric acid (300 cm^3), and washed thoroughly (by decantation) with water (5 dm^3) and dil aqueous ammonia (pH 7.0—8.0). The gel sludge was immediately treated with an aluminium chloride aqueous solution (0.2 M†, 50 cm^3 per the gel sludge equivalent to 10 g of dry weitht) for two days for aluminium ion doping. The pH of the supernatant of the mixture was kept at 7.0—8.0 by occasional adjustments with aqueous ammonia. The aluminium ion-doped gel sludge, isolated by decantation from the supernatant and precipitated aluminium hydroxide and washed with dil hydrochloric acid (pH 4.0), was divided into parts for imprinted (gel) catalysts and a control (gel) catalyst. A part of the doped gel was added to an acetone solution of a template (2×10^{-3} M of **1**, **2**, or **3**, 30 cm^3 per the gel sludge equivalent to 10 g of dry weight) and the pH of the supernatant of the mixture was adjusted to 4.0 with dil hydrochloric acid (pH 1.0). The mixture was allowed to stand for 2—5 days. For the control (gel) catalyst preparation, a template was omitted, other procedure being the same those as above mentioned. The gel, washed with dil hydrochloric acid (pH 4.0) and collected by filtration, was dried in a desiccator over calcium chloride for one day at room temperature and atmospheric pressure, and for further two days at reduced pressure of 25 mmHg.†† Finally, it was dried at 120—140 °C and the pressure of 3 mmHg, to give an "imprinted" (gel) catalyst. In the study on substrate specificity alteration, the two imprinted catalysts {**1**} and {**2**}, and one control catalyst without the imprinting were prepared in particular from the same origin of aluminium ion-doped silica gel preparation for exact comparison of the imprinting effects on the substrate specificity.

Acidic properties characterizations were carried out by methods of Benesi[10] with Hammett indicators for acid strength determination, and of Pines and Haag[11] with phenolphthalein for Lewis acid sites detection.[1]

Apparatus. The spectrophotometers used were the Hitachi 124 and Shimadzu UV-160. Triple-wavelength measurements were carried out with the latter using its internal data-processing program.

Kinetic Measurement. An appropriate amount of stock benzoic anhydride solution freshly prepared (1.5×10^{-2} M in acetonitrile) was diluted to 29 cm^3 with acetonitrile (final substrate concentration, $1—5\times10^{-3}$ M) in a glass-stoppered flask. To this solution was added 30 mg of a catalyst; the mixture was equilibrated at 30 °C for 10 min under gentle stirring. One cm^3 of stock 2,4-dinitrophenolate solution (5.0×10^{-3} M, prepared by dissolving 27.8 mg of potassium 2,4-dinitrophenolate and equivalent molar of 18-crown-6 in 25 cm^3 acetonitrile) was added in order to initiate the reaction under vigorous stirring. An aliquot, 3 cm^3, was taken out at proper intervals (2—3 min) and was filtered to remove off catalyst gel particles as quickly as possible, and the O.D. at 430 nm was recorded. When a triple-wavelength photometer was available, this filtration procedure was omissible by selecting wavelengths at 400, 430, and 500 nm; thereby, a more reliable determination was achieved than by the filtration procedure. First-order rate constants, k_{obs}, were calculated from the decrease in O.D. at 430 nm. In inhibition studies, the catalyst was equilibrated with an inhibitor in acetonitrile for 1 h at 30 °C; after the addition of the substrate solution, equilibration was continued for a further 5—10 min. The following procedures were the same as those mentioned before. Kinetic parameters, K_m and $k_{obs.max}$ (V_{max} in Michaelis-Menten kinetics) were obtained from Lineweaver-Burk plots using a least-squares method, and k_{cat} s were calculated from the $k_{obs.max}$ divided by the catalytic sites molarities obtained from a catalytic sites titration (see text).

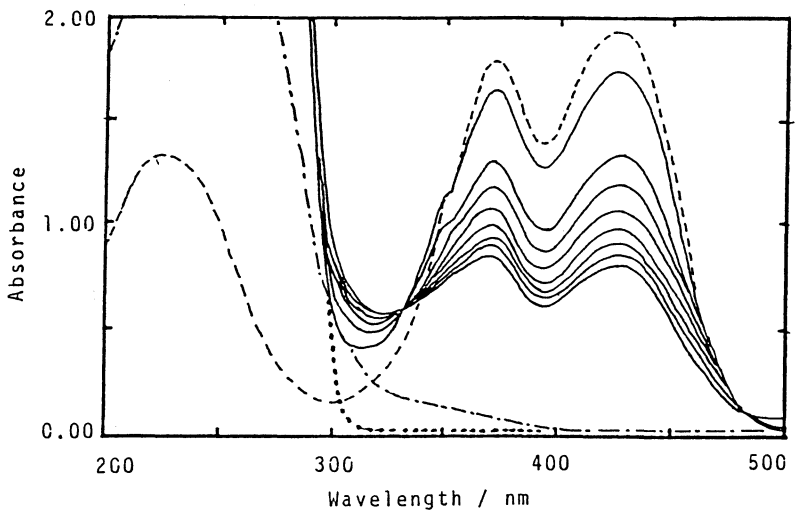


Fig. 1. Spectra changes in 2,4-dinitrophenolysis of benzoic anhydride by the footprint catalyst {**3**}.
——: reaction mixture, 4 min intervals. ----: nucleophile, 2,4-dinitrophenolate anion (1.67×10^{-4} M). ····: substrate, benzoic anhydride (1×10^{-3} M). —·—: product, 2.4-dinitrophenyl benzoate (ca. 1.67×10^{-4} M).

† 1 M=1 mol dm^{-3}.
†† 1 mmHg=133.322 Pa.

Results and Discussion

Photometric Assay of 2,4-Dinitrophenolysis. As can be seen in Fig. 1, the absorption spectra of 2,4-dinitrophenolate changed rapidly in the visible region (376, 430 nm) with the progress of the reaction; changes were evidently caused only by the catalytic 2,4-dinitrophenolysis, since no change was detected without a catalyst or substrate under the same reaction conditions.

Since no absorption of benzoic anhydride (substrate) or authentic 2,4-dinitrophenyl benzoate (product) was found in the region over 400 nm, the reaction rates could be simply determined through the O.D. decrease at 430 nm. After an initial slight curvature (within 8 min), linear semi-log plots of O.D. vs. time were usually observed over at least 1 half life, demonstrating that the reactions obey first-order kinetics with respect to the 2,4-dinitrophenolate concentration; the rate constants, $k_{obs.}$ s, were determined in the usual manner.

As a nucleophile, 2,4-dinitrophenolate was preferred to *p*-nitrophenolate, since the latter caused a too rapid *p*-nitrophenolysis of benzoic anhydride to determine the reaction rate, accompanying an uncatalyzed reaction in considerable quantities. The reverse reactions of the above-mentioned phenolyses, in which *p*-nitrophenyl benzoate or 2,4-dinitrophenyl benzoate were substrates and benzoate anion serves as a nucleophile[12] also caused a problem in that the release of phenolate anions was hindered by their adsorption on the gel surface.

As shown in Figs. 2 and 3 ([I])=0), the k_{obs} s obey Michaelis-Menten kinetics with respect to the anhydride substrates concentration. This fact suggests that

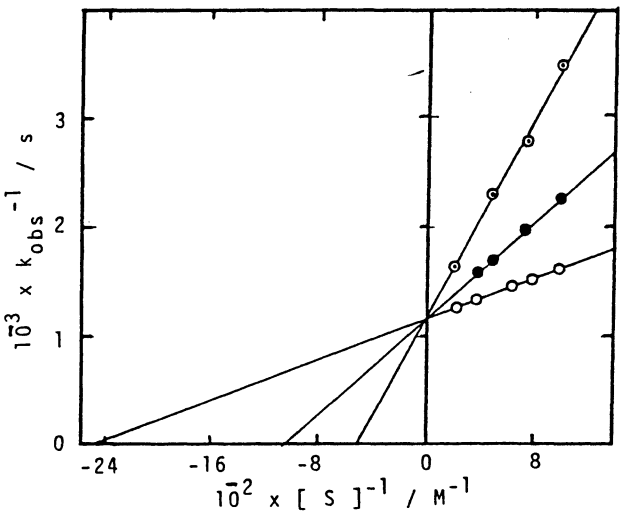


Fig. 2. Lineweaver-Burk plots for 2,4-dinitrophenolysis of benzoic anhydride catalyzed by the footprint catalyst {**3**}.
[S]: benzoic anhydride concentration. [I]: inhibitor **3** (Bz-NH-Bz) concentration. ○: [I]=0. ●: [I]=2.89×10^{-7} M. ◉: [I]=5.78×10^{-7} M. K_i=1.88×10^{-7} M.

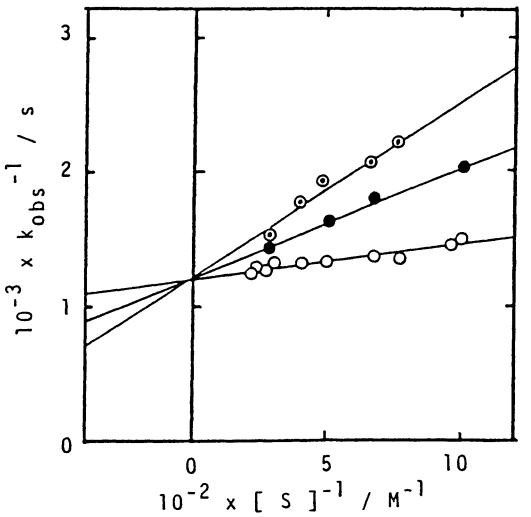
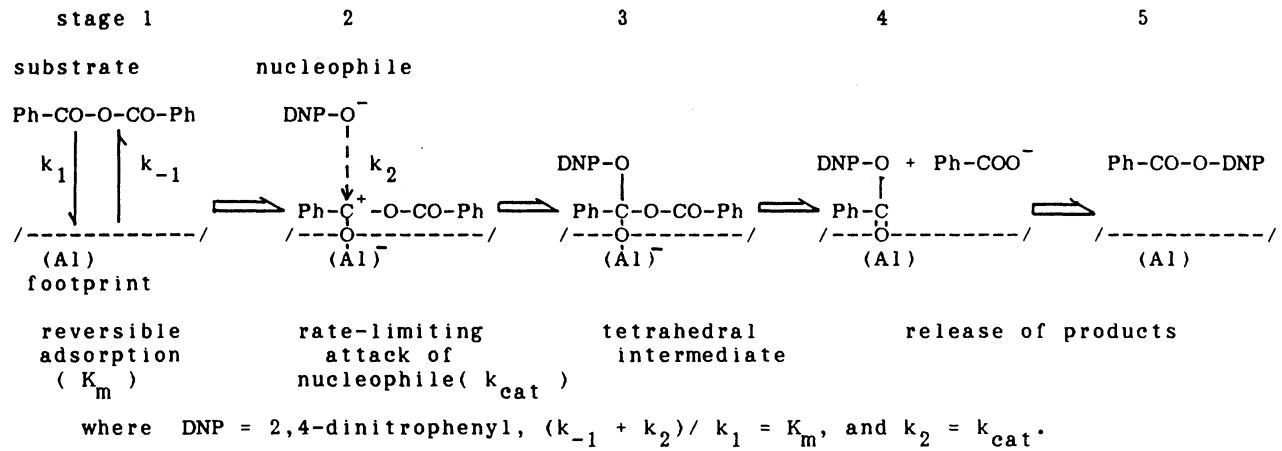


Fig. 3. Linewerver-Burk plots of footprint catalyst {**1**}.
[S]: substrate (benzoic anhydride) concentration. [I]: inhibitor **1**$^{-}$(Ph-SO_2-N^{-}-Bz) concentration. ○: [I]=0, ●: [I]=3.14×10^{-8} M, ◉: [I]=7.48×10^{-8} M. K_m=2.22×10^{-4} M, K_i=2.30×10^{-8} M, K_m/K_i=9.65×10^{3}.



Scheme 1. Catalytic process of dinitrophenolysis of benzoic anhydride.

the reactions proceed through a Rideal-Eley mechanism with a rate-limiting nucleophilic attack (Scheme 1) similar to the butanolysis of benzoic anhydride previously reported.[1] The rate law is also expressed as follows.

$$\text{rate} = \frac{k_{cat}[\text{catalyst}][\text{benzoic anhydride}][\text{2,4-dinitrophenolate}]}{K_m + [\text{benzoic anhydride}]}$$

All of the imprinted catalysts under investigation were confirmed to possess footprint catalytic sites by the criterion that they showed typical competitive inhibition by the original template molecules with huge K_m/K_i values (K_i is the dissociation constant of the inhibitor bound on a catalytic site), as shown in Figs. 2, 3, and 4. Additionally to note, conjugate bases of the template molecules were used as inhibitors in the latter two cases (Figs. 2 and 3) and were found to be more (10-fold) effective than the free bases. This fact demonstrates that conjugate bases are probably the intrinsic footprint marking species in the imprinting process.

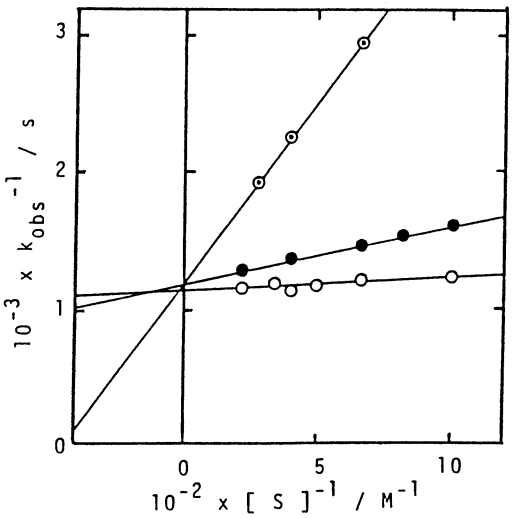


Fig. 4. Lineweaver-Burk plots of footprint cataltst {2}.
Substrate: *p*-toluic anhydride.
Inhibitor: 2⁻(CH_3-C_6H_4-SO_2-N^--CO-C_6H_4-CH_3).
○: [I]=0, ●: [I]=1.35×10^{-7} M, ◉: [I]=4.41×10^{-7} M.
K_m=7.85×10^{-5} M, K_i=2.45×10^{-8} M, K_m/K_i=3.2×10^3.

Regarding the acidic properties of the catalysts, all of the catalysts were positive to some Hammett indicators (Neutral Red, pK_a +6.8; Methyl Red, pK_a +4.8; 4-(phenylazo)diphenylamine, pK_a +1.5) and to phenolphthalein for Lewis acid (purple), which were the same as those reported previously.[1]

The kinetic parameters are summarized in Table 1, in which data of the butanolysis[1] analyzed by the potentiometric titration are also cited for a comparison. As can be seen, remarkable differences, 3 orders of magnitude, can be observed in the binding steps (K_m s, K_i s) between the 2,4-dinitrophenolysis and the butanolysis, These differences are presumably caused by the poor solvating ability of aprotic acetonitrile,[13] as compared with that of protic 1-butanol, i.e., all the substrate, inhibitors and footprint catalytic sites[14] might be less solvated or more "naked" in acetonitrile than in 1-butanol. The binding steps, thereby, would be strikingly enhanced. The huge rate enhancements observed in catalytic steps (k_{cat} s) (5 order of magnitude), are also explainable by the strengthened nucleophilicity of "naked" 2,4-dinitrophenolate anions solublized into dipolar acetonitrile with the aid of a crown ether.[15-17] The two effects mentioned above result in an enormous rate enhancement for the overall reaction (k_{cat}/K_m), 7 orders of magnitude.

By the application of this enhanced reaction in acetonitrile to an assay of a catalysis, a photometric method is successfully established as required, i.e., the butanolysis method needs 2.26 g of the substrate, 700 mg of a catalyst, and 120 min of reaction time at 55 °C per a measurement, whereas the new dinitrophenolysis method requires only 6.8 mg of the substrate, 30—50 mg of a catalyst, and 25 min at 30 °C per measurement.

Table 1. Kinetic Parameters of the Reactions

Catalyst imprinted with { }	k_{cat}[cat]	k_{cat}[a]	K_m	k_{cat}[cat]/K_m	k_{cat}/K_m	K_1	K_m/K_1
	s^{-1}	$M^{-1}s^{-1}$	M	$M^{-1}s^{-1}$	$M^{-2}s^{-1}$	M	
2,4-Dinitrophenolysis of benzoic anhydride in acetonitrile at 30 °C.[b]							
{Ph-SO_2-NH-Bz}[c]	2.9×10^{-2}	1.5×10^3	4.5×10^{-4}	6.5×10	3.2×10^6	1.8×10^{-7}	2580
{Bz-NH-Bz}[d]	2.9×10^{-2}	2.8×10^3	4.1×10^{-4}	7.1×10	6.9×10^6	1.9×10^{-7}	2220
Control	2.8×10^{-2}		1.8×10^{-3}	1.6×10		No inhibition	
Butanolysis in 1-butanol-benzene (30:70, v/v) at 55 °C.[e]							
{Ph-SO_2-NH-Bz}	5.4×10^{-7}		2.0×10^{-1}	2.7×10^{-6}		2.0×10^{-4}	970
{Bz-NH-Bz}	4.9×10^{-7}		1.6×10^{-1}	3.0×10^{-6}		Not determined	
{Bz-NH-Bz}	4.8×10^{-7}		1.3×10^{-1}	3.7×10^{-6}		4.7×10^{-5}	2770
Control 1	1.6×10^{-6}		1.7	9.8×10^{-7}		No inhibition	
Control 2	5.7×10^{-7}		1.3	4.6×10^{-7}		No inhibition	

a) Calculated with the molarities of catalytic sites per g determined by titration (see text).
b) This study. c) Molarity of catalytic sites, 2.01×10^{-5} mol per g. d) 1.03×10^{-5} mol per g.
e) Data from previous paper,[1] of which V_{max} values were devided by initial 1-butanol concentration and normalized per second and per g catalyst.

Catalytic Sites Titration by Pyridine Poisoning. Figure 5 demonstrates typical Lineweaver-Burk plots for an irreversible poisoning of the catalytic sites of {3} with pyridine. Similar plots for the poisoning have also been observed with the catalysts, {1}, {2} and control (plots are not shown). This poisoning by pyridine was applied to a titration method for a molarity of catalytic sites which is a requisite for k_{cat} calculation. As shown for the example in Fig.6, the amounts of catalytic sites of {3} could be determined from extrapolations of the linear k_{obs} vs. pyridine concentration plots to intercept on abscissa, since pyridine showed no other effect on 2,4-dinitrophenolysis. The titration was so accurate that the amounts of catalytic sites per g of a catalyst {3} (0.99—1.03×10^{-5} mol) obtained from 30 and 40 mg of the samples were practically identical within the experimental error. Similar titration by pyridine poisoning for the butanolysis of benzoic anhydride was rather difficult because the excess pyridine induced a general base catalysis which promoted butanolysis. As can be seen in Fig. 7,[18] where k_{obs} vs. pyridine plots had a break, the number of catalytic sites (6.92×10^{-5} mol per g) calculated from the ambiguous break point was not so reliable in accuracy as that from the dinitrophenolysis method. However, the finding that estimates of the same orders of the catalytic sites per g have been found from both butanolysis and dinitrophenolysis supports the hypothesis that these two reactions are catalyzed by the same catalytic sites, though remarkable differences have been observed in the kinetic parameters.

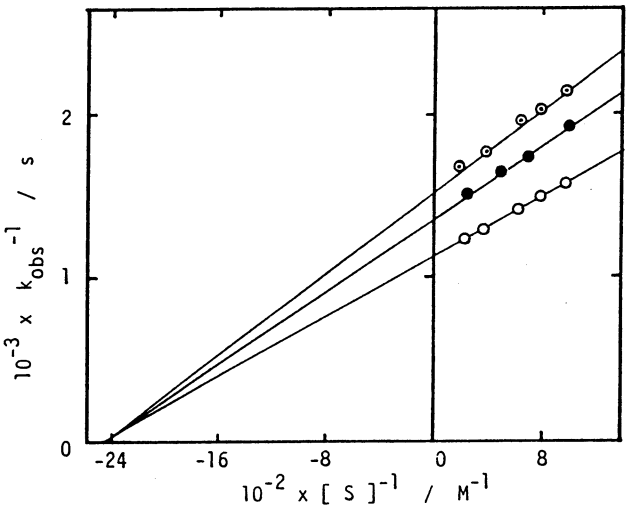


Fig. 5. Poisoning of the footprint catalyst {3} by pyridine; irreversible inhibition.
[S]=benzoic anhydride concentration. [I]=pyridine concentration. ○: [I]=0. ●: [I] 1.28×10^{-6} M. ◉: [I]=2.56×10^{-6} M.

It is worth mentioning here that the magnitude of the molarity of footprint catalytic sites, e.g., 2.0×10^{-5} mol per g for a catalyst {1}, is so large that the footprints cover about 2—3 percent of the surface of Kieselgel, assuming that the surface area of the gel is 500 m^2 per g and that of the template **1** is about 100 Å^2 per molecule. This larfge coverage by footprints would be effectively applicable to the design of specific adsorbents for a kind of affinity chromatography.

Effect of 2,6-Dimethylpyridine (Noninhibition). Figure 6 also shows that the addition of 2,6-dimethylpyridine gives rise to a noninhibitory effect on catalytic sites. This finding evidently confirms the idea that the footprint catalytic sites possess Lewis acid

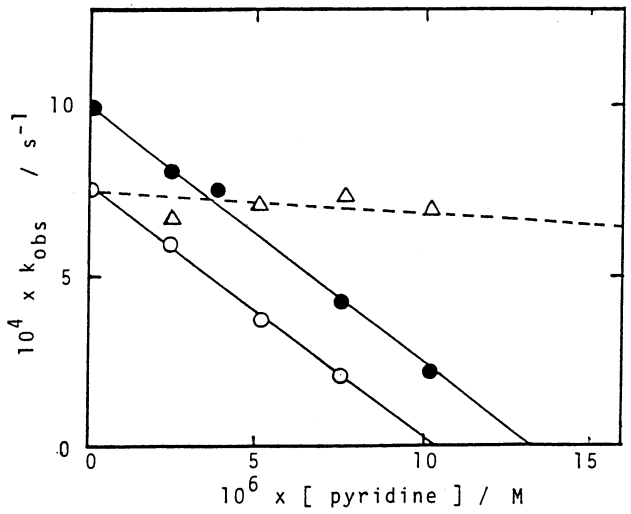


Fig. 6. Catalytic sites titration of the footprint catalyst {3} by pyridine poisoning in 2,4-dinitrophenolysis of benzoic anhydride.
[benzoic anhydride]: 2.5×10^{-3} M. ○: 30 mg catalyst. titre; 1.03×10^{-5} mol per g. ●: 40 mg catalyst. Titre; 9.96×10^{-6} mol per g. △: 2,6-dimethylpyridine to 30 mg catalyst (no inhibitory effect).

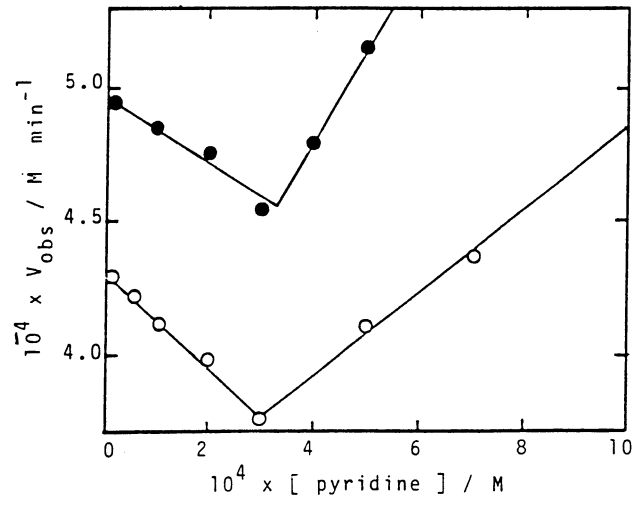


Fig. 7. Catalytic sites titration of a footprint catalyst {3} and a control catalyst by pyridine poisoning in butanolysis of benzoic anhydride. (Data from Ref. 18 referred for comparison.) Assay procedure was the same as descrelbed in Ref. 1.
○: control catalyst. titre; 6.00×10^{-5} mol per g. ●: catalyst{3}. titre; 6.92×10^{-5} mol per g.

sites, as previously discussed, since if the catalytic sites are Brønsted acid sites, they should lose their catalytic activities by neutralization with 2,6-dimethylpyridine. However, Lewis acid sites were still active because they cannot form acid-base complexes with 2,6-dimethylpyridine through steric hindrance to two methyl groups. It should be noted that a footprint catalyst and 2,6-dimethylpyridine forms an unusual reaction medium in which an acid and a base cannot act on each other like the case of "wolf and lamb" reaction,[19] and its potential utility as a concerted acid-base catalysis might be promising.

Alteration of Substrate Specificities. In order to distinguish the footprint effects of different catalytic sites on the substrate specificities, phthalic anhydride is not adequate, though it has been used once as a substrate in a previous study.[1] This is because its much higher reactivity than benzoic anhydride has covered (by leveling off) the difference in the catalytic effects, which had been observed in the catalysis for benzoic anhydride, between the imprinted catalytic sites (of {1} and {2}) and the native Lewis acid sites of the control catalyst. Consequently, benzoic anhydride and its *p*-, *o*-, *m*-methyl derivatives are selected here as a set of substrates to be used to compare the catalytic activities, since they have similar original reactivities, and also have similar binding capabilities due to their weak hydrophobic interactions with a silicate surface of the catalyst.

The four anhydride substrates were subjected to catalyzed reactions by the three catalysts, {1}, {2} and the control catalyst, to give twelve Lineweaver-Burk plots. They showed fairly good plots (see correlation coefficients r in Table 2) except one (r=0.6362) for *m*-toluic anhydride by catalyst {1} for uncertain reasons. Some typical plots are shown in Figs. 8 and 9.

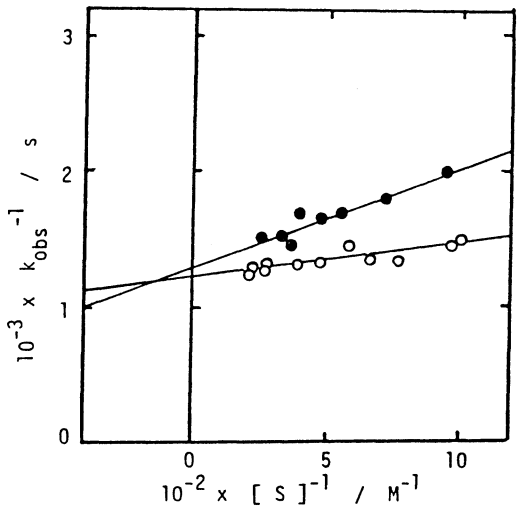


Fig. 8. Catalytic effects of footprint catalyst {1} upon benzoic anhydride and *o*-toluic anhydride.
[S]: anhydride concentration. ○: benzoic anhydride. ●: *o*-toluic anhydride.

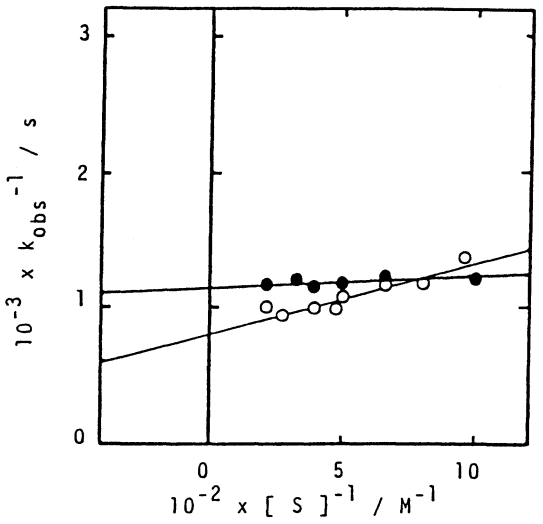


Fig. 9. Catalytic effects of footprint catalyst {2} upon benzoic anhydride and *p*-toluic anhydride.
○: benzoic anhydride. ●: *p*-toluic anhydride.

In Table 2 are summarized the kinetic parameters for the catalyzed reactions. Since the four anhydride substrates have still different reactivities in Lewis acid catalyzed reactions, the kinetic parameters have been normalized by dividing by the corresponding values of the control catalyst. With the aid of these normalized values, the characteristic catalytic effects of the footprint catalytic sites can be revealed separately from the original specificities of the control catalyst. The values of k_{cat}/K_m are reasonable to express and compare the substrate specificities of catalysts, as often used in enzyme kinetics.[20] The normalized k_{cat}/K_m values, otherwise, may represent "substrate specificity enhancement," a factor of alteration of the original specificity of "native" catalytic sites of the control catalyst by imprinting.

As can be seen in Table 2, remarkable differences in specificities exist between catalysts {1} and {2}. Though they have originated from the same aluminum ion-doped silica gel preparation, the former prefers benzoic anhydride (normalized k_{cat}/K_m=2.49) to other anhydrides (0.55—0.82), whereas the latter prefers *p*-toluic anhydride (3.97) to other anhydrides (1.04—1.26). These significant alterations in specificities are not brought about by changes in k_{cat} values (normalized values of 0.89—1.25 for catalyst {1}, 1.05—1.15 for {2}), but mainly come from changes in K_m values, owing to the exact molecular recognition of footprint catalytic sites.[2] Consequently, catalyst {1} shows the largest affinity (normalized K_m=0.36) to benzoic anhydride, possibly due to an inclusion effect of footprint sites through a best fitting with maximal interactions, and less affinities (1.46—1.79) to others due to exclusion effects of footprints through a steric hindrance to the methyl group of the substrates. However, catalyst {2} displays the largest affinity (0.31) to

Table 2. Kinetic Parameters and Substrate Specificities

Catalysts	Kinetic parameters	Substrate anhydrides			
		Benzoic	*p*-,	*m*-,	*o*-Toluic
Cat.{**1**}[c]	n[a]	11	14	15	8
	r[b]	0.9180	0.8447	0.6362	0.9286
	$K_m/10^{-4}$M	2.22	4.54	5.13	5.90
	(K_m)[d]	(0.36)[d]	(1.79)	(1.46)	(1.74)
	$k_{cat}/10^3\,M^{-1}s^{-1}$	1.07	0.97	1.07	1.04
	(k_{cat})	(0.89)	(1.25)	(1.20)	(0.95)
	$k_{cat}/K_m/10^6M^{-2}s^{-1}$	4.81	2.15	2.08	1.77
	(k_{cat}/K_m)	(2.49)	(0.70)	(0.82)	(0.55)
Cat. {**2**}[e]	n	8	6	8	5
	r	0.9406	0.7971	0.8805	0.9816
	$K_m/10^{-4}$M	6.85	0.79	3.97	2.97
	(K_m)	(1.09)	(0.31)	(1.12)	(0.87)
	$k_{cat}/10^3\,M^{-1}s^{-1}$	1.39	0.96	1.26	1.15
	(k_{cat})	(1.15)	(1.23)	(1.41)	(1.05)
	$k_{cat}/K_m/10^6\,M^{-2}s^{-1}$	2.02	12.19	3.17	3.85
	(k_{cat}/K_m)	(1.04)	(3.97)	(1.26)	(1.20)
Control[f]	n	4	4	4	4
	r	0.9925	0.9961	0.9581	0.8992
	$K_m/10^{-4}$M	6.28	2.53	3.52	3.40
	$k_{cat}/10^3\,M^{-1}s^{-1}$	1.21	0.77	0.89	1.09
	$k_{cat}/K_m/10^6M^{-2}s^{-1}$	1.93	3.07	2.52	3.21
		(1.00)	(1.00)	(1.00)	(1.00)

a) Numbers of data points on Lineweaver-Burk plots. b) Correlation coefficients. c) Molarity of catalytic sites, 2.58×10^{-5} mol per g. d) Figures in parentheses mean normalized values obtained by dividing with the corresponding values of the control catalyst. e) 3.08×10^{-5} mol per g. f) 2.93×10^{-5} mol per g.

p-toluic anhydride and also less affinity (1.0) to benzoic anhydride, though the latter can be bound onto the footprints of catalyst {**2**} without any steric hindrance. This fact suggests that the catalyst {**2**} can differentiate not only an inert methyl group in the *p*-, *m*-, or *o*-psition through the inclusion or exclusion effects, but also can recognize even a lack of the methyl group through a slight difference in their weak hydrophobic interactions.

Thus, the exact "complementarity" of the footprints to a template is clearly demonstrated to act effectively on substrate specificities in a catalytic reaction.

Conclusion

Substrate specificities of silica(alumina) catalysts can be inductively altered by the authors' imprinting method with a choice of appropriate template molecules, which would be the fundamental base of a practical tailor-made catalyst design.

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