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Application of Hydrogen Storage Alloy to Remove Protecting Groups

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Hydrogen storage alloys are capable of charging and discharging hydrogen in temperature-dependent manner, and are employed as catalysts for hydrogenation and dehydration. In water, $CaNi_5$ removed protecting groups such as benzyloxycarbonyl, benzyl ester, benzyl ether and nitro groups without any side reactions, and the reaction time was highly dependent on the hydrogen pressure of the reaction system. In organic solvents such as dioxane and EtOAc the deprotection reaction did not proceed. Methanol (MeOH) was applicable, but the reaction in MeOH required a longer reaction time than in water. A mixture of water and MeOH accelerated the reaction depending on the water content. Although the mechanism of the catalytic hydrogenation with $CaNi_5$ in water and/or MeOH is uncertain, the reaction between Ca and water or MeOH seems to be of critical importance.

Keywords hydrogen storage alloy; catalytic hydrogenation; deprotection; peptide synthesis; $CaNi_5$

Peptide synthesis is important in chemistry, pharmacology and agriculture. We have attempted to develop a new procedure for deprotection by catalytic hydrogenation, which would be suitable for industrial purposes. A variety of procedures have been used to remove protecting groups employed in peptide synthesis, for example, acid treatment for removal of the *tert*-butyloxycarbonyl group (Boc),[1] *p*-toluenesulfonyl group[2] and *p*-methoxybenzyloxycarbonyl group,[3] alkali treatment for the 9-fluorenylmethyloxycarbonyl group[4] and methylsulfonylethyloxycarbonyl group,[5] and catalytic hydrogenation for the benzyloxycarbonyl group (Z),[6] benzyl ester (OBzl),[7] benzyl ether (Bzl)[8] and nitro group (NO_2).[9] Catalytic hydrogenation is favorable for the removal of protecting groups, because the reaction is performed under mild conditions without damaging the peptide.[10] Precious metals such as Pt and Pd are widely used as catalysts for the catalytic hydrogenation,[11] but are expensive for commercial use. Raney catalysts, such as Raney Ni and Raney Co, are also used as catalysts for hydrogenation, and are used industrially for production of sugar alcohol[12] or hydrogenation of unsaturated fatty acids,[13] but these Raney catalysts do not catalyze the removal of protecting groups in peptide synthesis.

Alloys such as La–Ni, La–Ni–Mg, Mg–Ni, Ti–Fe, Zr–Co and Zr–Co–Ni are hydrogen storage alloys capable of charging and discharging hydrogen in a temperature-dependent manner.[14] Much attention has been paid to these alloys because of their application to heat-related instrumentation by making use the endothermic and exothermic reactions associated with hydration and de-hydration.[15] They have a high hydrogen storage capacity and discharge highly pure hydrogen (99.999%).[16] The hydrogen storage alloys containing Ni are employed as catalysts for hydrogenation, including reduction of alkenes, alkanes and aldehydes[17] and also for dehydrogenation of cyclohexane[18] and alcohols.[19] To extend the application of hydrogen storage alloys, we examined the usefulness of $CaNi_5$ as a catalyst to remove peptide protecting groups such as Z , OBzl, Bzl and NO_2, and investigated the effects of various reaction conditions.

Experimental

Materials $CaNi_5$ was purchased from Mitsubishi Seiko Co. Ltd. (Tokyo), Raney Ni and palladium carbon (Pd–C) containing 5% Pd were from Wako Pure Chemical Industries (Osaka), and all amino acid derivatives and aspartame (authentic sample) were from Peptide Institute (Osaka). The precursor of aspartame, Z–Asp–Phe–OMe, was enzymatically synthesized according to the method described by Isowa *et al.*[20]

Catalytic Hydrogenation with $CaNi_5$ Lumps of $CaNi_5$ were crushed and heated to 350 °C under low hydrogen pressure (below 5 kg/cm^2) in an enclosed vessel (1.2 l), then cooled to 10 °C. The procedure of heating and cooling at temperature ranges from 10 to 120 °C under a hydrogen pressure below 9 kg/cm^2 was repeated five times to achieve a particle diameter of $CaNi_5$ between 50 and 500 μm. The pulverized $CaNi_5$ was cooled with liquid nitrogen to -100 °C for 30 min under a hydrogen pressure of 9 kg/cm^2. Then the reaction vessel was evacuated, a degassed solution containing a starting material was fed into the vessel, and the reaction mixture was heated to a given temperature. Hydrogen was introduced into the reaction vessel to adjust the hydrogen pressure, if necessary. The reaction was monitored by thin layer chromatography (TLC) (Art. 5631, Merck) with a densitometer (Chromatoscanner CS-930, Shimadzu), or by amino acid analysis with an automatic amino acid analyzer (Hitachi model 835).

Catalytic Hydrogenation with Pd–C or Raney Ni Catalytic hydrogenation with Pd–C or Raney Ni was performed in the conventional way, using a reaction vessel (1.2 l). The progress of the reaction was monitored by TLC.

Conversion of Z–Asp–Phe–OMe to Aspartame In the reaction vessel (1.2 l), 50 g of fine $CaNi_5$ powder was charged with hydrogen at -100 °C for 30 min under the hydrogen pressure of 9 kg/cm^2. Then the reaction vessel was evacuated, and a water–methanol (MeOH) (1 : 2, v/v) medium containing 2.14 g (5 mmol) of Z–Asp–Phe–OMe was fed into the reaction vessel, and stirred at 40 °C for 3 h under a hydrogen pressure of 9 kg/cm^2. To separate the alloy from the reaction mixture, the reaction mixture was passed through a microfilter Chromato Disc 25A with pore size of 0.45 μm (GL Science, Tokyo). The filtrate was evaporated and the residue was suspended in MeOH. MeOH-insolubles were removed through filtration with Filter Paper 5A (Advantec, Tokyo), then the filtrate was evaporated. Finally 1.35 g of white powder was recovered.

Determination of Minerals Minerals released from $CaNi_5$ were determined by inductive coupled plasma atomic emission spectrometry (JICP-PSW3000UV, Leeman Labs).

Observation of $CaNi_5$ with Electron Microscope $CaNi_5$ was observed with an electron microscope, according to the method described by Hayat.[21]

Results and Discussion

Characteristics of Hydrogen Discharge by $CaNi_5$ As shown in Fig. 1, hydrogen-charged $CaNi_5$ released hydrogen in proportion to the heating temperature up to 100 °C and reached saturation at 13.58 kg/cm^2 when the 1.2 l reaction vessel was employed. Figure 2 shows the relationship between the amount of $CaNi_5$ and equilibrated hydrogen pressure at 40 °C in the reaction vessel (volume: 1.2 l). The pressure of hydrogen discharged from 10 g of $CaNi_5$ was below 0 kg/cm^2, because the inside of the reaction vessel was initially under vacuum. Therefore, hydrogen pressure in the reaction vessel increased with the amount of $CaNi_5$. At 40 °C the pressure of hydrogen discharged from 100 g of $CaNi_5$ was 4 kg/cm^2.

Removal of Protecting Groups with $CaNi_5$ in Water Table I summarizes the reaction time required to remove completely the protecting groups Z, OBzl, Bzl and NO_2 from the corresponding amino acid derivatives by catalytic hydrogenation in water. For comparison, Raney Ni and Pd–C were used to remove the Z group from Z–Ala–OH. The reaction time indicated in Table I means the time required for complete deprotection. In water $CaNi_5$ removed all protecting groups within 120 min. Side reactions due to catalytic hydrogenation with $CaNi_5$, such as hydrogenation of the phenyl group (Phe)[22] or indole ring (Trp)[23] and desulfuration (Met),[24] were not detectable. The relatively longer reaction time required to remove the Z group from Z–Trp was attributable to the poor solubility of Z–Trp. The removal of Z from Z–Ala–OH using Pd–C was completed within 10 min; on the other hand, Raney Ni did not catalyze the reaction.

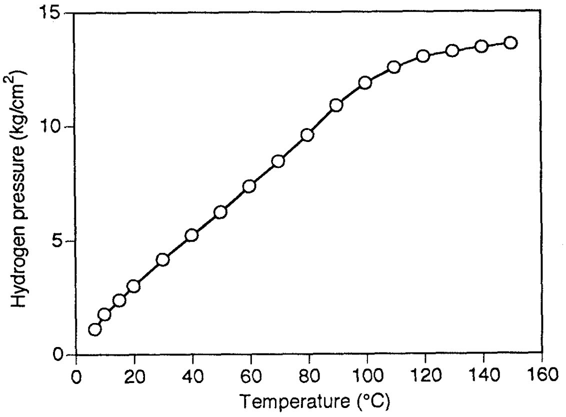

Fig. 1. Hydrogen Discharge from $CaNi_5$

$CaNi_5$: 100 g. Reaction vessel: 1.2 l.

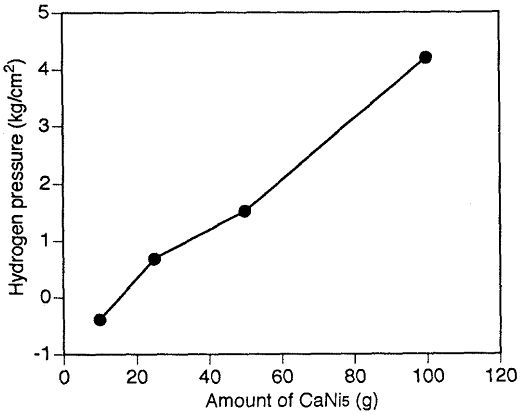

Fig. 2. Relationship between Amount of $CaNi_5$ and Equilibrated Hydrogen Pressure at 40°C in Water

The volume of the reaction vessel was 1.2 l.

The unit surface areas of $CaNi_5$ and Raney Ni were 0.0639 m^2/g[25] and 50—110 m^2/g,[26] respectively. Thus the difference in surface area does not explain the superior catalytic ability of $CaNi_5$ over Raney Ni. Presumably its hydrogen storage ability enabled $CaNi_5$ to achieve the deprotection reactions, that is, hydrogen in atomic form[27] discharged from $CaNi_5$ may be critical for the catalytic hydrogenation.

Effects of Organic Solvents on Catalytic Hydrogenation with $CaNi_5$ Table II shows the reaction time to remove the Z group in organic solvents; this means the time required for complete deprotection. The reaction time with $CaNi_5$ was much longer in methanol than in water, and the reaction hardly proceeded in ethyl acetate (EtOAc) or dioxane. The low catalytic ability of $CaNi_5$ in organic solvents is unfavorable for use in peptide synthesis, because

Table I. Catalytic Hydrogenation of Amino Acid Derivatives by $CaNi_5$ in Water at 40 °C

Catalyst (g)	Weight (g)	Sample[a]	Hydrogen pressure (kg/cm^2)	Reaction time[b] (min)
$CaNi_5$	100	Z–Ala–OH	3.0	20
$CaNi_5$	100	Z–Met–OH	4.7	<5
$CaNi_5$	100	Z–Trp–OH	4.3	120
$CaNi_5$	100	H–Phe–OBzl	9.0[c]	5
$CaNi_5$	100	H–Ser(Bzl)–OH	9.0[c]	90
$CaNi_5$	100	H–Arg(NO_2)–OH	9.0[c]	60
Pd–C[d]	25	Z–Ala–OH	9.0[c]	10
Raney Ni	50	Z–Ala–OH	9.0[c]	—[e]

a) The starting material (2 g) was dissolved in 398 g of water. *b*) Time for completing the reaction. *c*) Pressurized with hydrogen. *d*) Containing 5% (w/w) Pd. *e*) No reaction.

Table II. Effect of Solvent on Removal of Protecting Groups with $CaNi_5$[a]

Catalyst	Solvent	Starting material[b]	Hydrogen pressure (kg/cm^2)	Temperature (°C)	Reaction time[c] (min)
$CaNi_5$	MeOH	Z–Ala–OH	1.0	25	>720
$CaNi_5$	MeOH	Z–Ala–OH	9.0[d]	40	600
$CaNi_5$	Dioxane	Z–Ala–OH	3.7	40	±[e]
$CaNi_5$	Dioxane	Z–Phe–OH	3.5	40	—[f]
$CaNi_5$	EtOAc	Z–Ala–OH	2.4	40	—[f]
Pd–C	MeOH	Z–Ala–OH	9.0[d]	40	<5
Raney Ni	MeOH	Z–Ala–OH	9.0[d]	40	—[f]

a) 100 g of $CaNi_5$ was used in each reaction. *b*) Each starting material (2 g) was dissolved in 298 g of solvent. *c*) Required time for completely deprotection. *d*) Pressurized with hydrogen. *e*) Reaction product was detectable on TLC. *f*) No reaction.

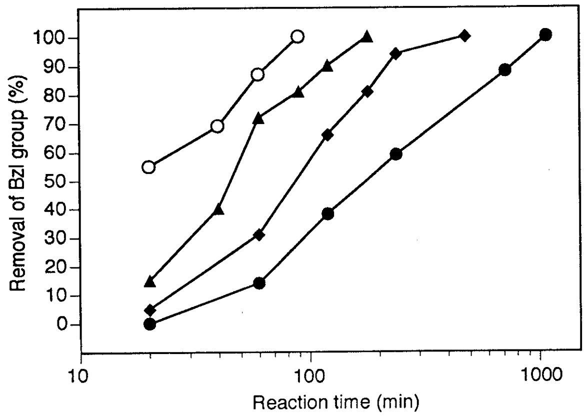

Fig. 3. Removal of Bzl Group by CaNi$_5$ in Water–MeOH Media

○, in water; ▲, in 12% MeOH; ◆, in 50% MeOH; ●, in 100% MeOH Boc–Ser(Bzl)–OH (2 g) was subjected to catalytic hydrogenation with 100 g of CaNi$_5$ in 498 g of water–MeOH media under the hydrogen pressure of 9 kg/cm^2 at 40°C.

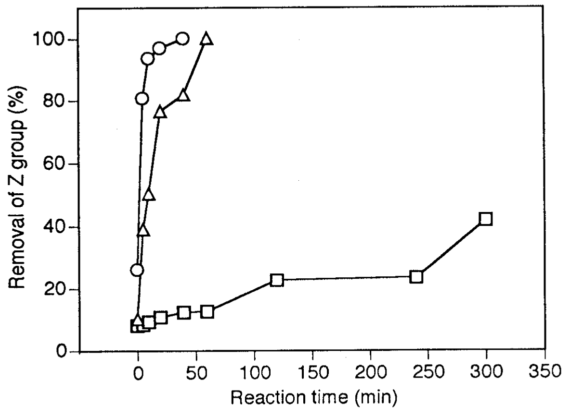

Fig. 4. Dependence of Reaction Time on Amount of CaNi$_5$ and Hydrogen Pressure

○, CaNi$_5$ 50 g (−0.4 kg/cm^2); △, CaNi$_5$ 25 g (0.63 kg/cm^2); □, CaNi$_5$ 10 g (1.52 kg/cm^2). Z–Ala–OH (2 g) was subjected to catalytic hydrogenation in 398 g of water at 40°C.

protected amino acids or peptides generally have poor solubility in water. Figure 3 shows the results of catalytic hydrogenation with CaNi$_5$ to remove the Bzl group in aqueous-MeOH media. Initially Boc–Ser(Bzl)–OH was suspended in an aqueous-MeOH medium because of its poor solubility in water, and the reaction mixture gradually became clear as the reaction proceeded. The reaction time was reduced with increase in water content in the media. When a compound insoluble in water is subjected to catalytic hydrogenation with CaNi$_5$, water–MeOH medium in a suitable mixing ratio should be employed to accelerate the reaction. If the starting material is insoluble even in MeOH, catalytic hydrogenation with CaNi$_5$ will be difficult.

As an example, 2.14 g of Z–Asp–Phe–OMe was converted to aspartame with 50 g of CaNi$_5$ in 50% MeOH under the hydrogen pressure of 9 kg/cm^2 at 40 °C. The converting reaction was completed within 180 min, resulting in 1.35 g (92% yield) of aspartame without formation of by-products. The reaction time and yield were comparable with those of conventional methods.[28]

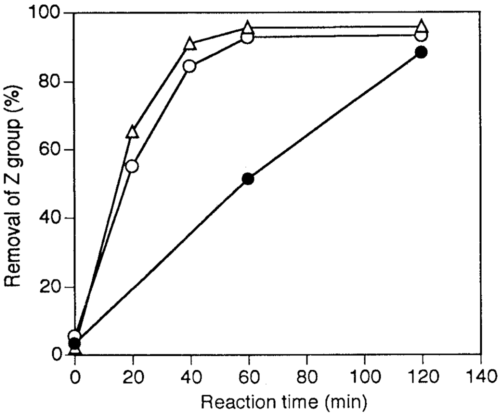

Fig. 5. Dependence of Reaction Time on Hydrogen Pressure and Temperature

○, at 25°C under 1.3 kg/cm^2; △, at 40°C under 1.3 kg/cm^2; ●, at 25°C under 0.9 kg/cm^2. Z–Val–OH (2 g) was subjected to catalytic hydrogenation with 100 g of CaNi$_5$ in 398 g of MeOH.

Table III. Metal Ions Released from CaNi$_5$[a)]

Metal ion	Content (mg) in 400 g of dispersion
Ca^{2+}	27.0
Ni^{2+}	0.05

a) 100 g of CaNi$_5$ was stirred in 398 g of water at 40°C for 18 h.

Effects of Amount of CaNi$_5$, Hydrogen Pressure and Temperature on Reaction Time Figure 4 shows the relationship between the amount of CaNi$_5$ and the reaction time required to remove the Z group. Since the pressure of hydrogen discharged from 10 g of CaNi$_5$ was below 0 kg/cm^2, the removal of the Z group was incomplete; only 40% was removed at 300 min. On the other hand, rapid reaction occurred when 50 g of CaNi$_5$ was used. Since the hydrogen pressure of the reaction system increased with increasing amount of CaNi$_5$, the rate of reaction seemed to be controlled by the hydrogen pressure. The effects of reaction temperature and hydrogen pressure on the reaction time in MeOH are shown in Fig. 5. At 25 °C the pressure of hydrogen discharged from 100 g of CaNi$_5$ was 1.3 kg/cm^2 and 95% of the Z group was removed within 60 min. When the hydrogen pressure was reduced to 0.9 kg/cm^2 at 25 °C, 120 min was required to complete the reaction. The effect of temperature was negligible at 1.3 kg/cm^2 hydrogen pressure. These findings suggested that the reaction catalyzed by CaNi$_5$ did not depend on the reaction temperature, but rather on the hydrogen pressure, in other words, the amount of the alloy. Compared with Pd–C, a larger amount of CaNi$_5$ was required to remove the protecting groups because the alloy is required to act as a hydrogen supplier.

Change of CaNi$_5$ Caused by Water Table III shows the metal ions released from CaNi$_5$ into water; 27 mg of Ca^{2+} was detected in the reaction mixture containing 100 g of CaNi$_5$, whereas the level of Ni^{2+} was very low. The pH of the suspension containing CaNi$_5$ was 12, probably due to $Ca(OH)_2$ derived from the reaction between Ca and H_2O. Figure 6 shows the surface of CaNi$_5$ observed through an electron microscope. Pulverization cracked the alloy, exposing fresh surfaces. (Fig. 6 A, B). After catalytic

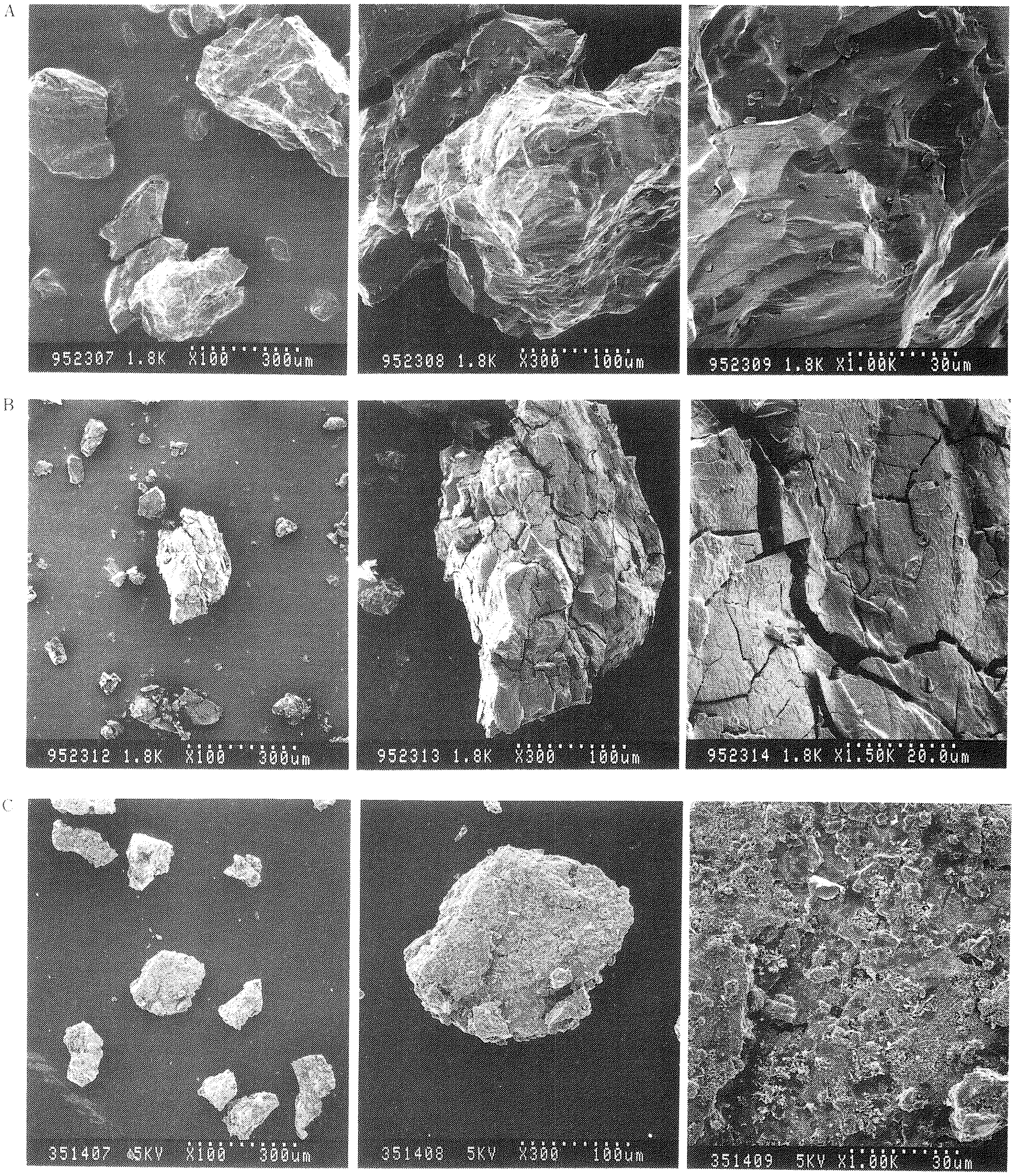

Fig. 6. $CaNi_5$ Observed under an Electron Microscope

A, before pulverization; B, after pulverization; C, after hydrogenation in water.

hydrogenation in water, the surface of $CaNi_5$ was changed significantly (Fig. 6 C), presumably owing to the release of Ca^{2+} ion from the alloy. MeOH was the only organic solvent usable for the reaction with $CaNi_5$. Water and MeOH react with Ca to form $Ca(OH)_2$ and $Ca(OCH_3)_2$, respectively, and generate hydrogen on the surface of the alloy, while EtOAc and dioxane do not. It seems that the reaction between Ca and water or MeOH plays an important role in the catalytic hydrogenation. Possible reasons for the solvent specificities of the catalytic hydrogenation are: (i) the generated hydrogen derived from the reaction of Ca with water and/or MeOH contributes to the reaction; (ii) release of Ca from the alloy erodes $CaNi_5$ and increases the surface area, which enhances the catalytic ability of the alloy; and (iii) the erosion of $CaNi_5$ exposes Ni at the surface of the alloy, leading to enhancement of the catalytic ability. However, the actual mechanism is not yet known.

$CaNi_5$ is an available catalyst for removal of protecting groups in peptide synthesis and seem to be applicable for industrial purpose because its price is presumably lower than that of the Pd catalysts. Before hydrogen storage alloy is employed in practical peptide synthesis, however, further studies are required to elucidate the mechanism of the catalytic hydrogenation with the alloy and its solvent specificity.

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