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RESEARCH ARTICLE

Factors affecting color strength of printing on film-coated tablets by UV laser irradiation: TiO_2 particle size, crystal structure, or concentration in the film, and the irradiated UV laser power

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Abstract

Aim: The purpose of this article is to study factors affecting color strength of printing on film-coated tablets by ultraviolet (UV) laser irradiation: particle size, crystal structure, or concentration of titanium dioxide (TiO_2) in film, and irradiated UV laser power.

Methods: Hydroxypropylmethylcellulose films containing 4.0% of TiO_2, of which BET particle sizes were ranging from 126.1 to 219.8 nm, were irradiated 3.14 W of UV laser at a wavelength 355 nm to study effects of TiO_2 particle size and crystal structure on the printing. The films containing TiO_2 concentration ranging from 1.0 to 7.7% were irradiated 3.14 or 5.39 W of the UV laser to study effect of TiO_2 concentration on the printing. The film containing 4.0% of TiO_2, was irradiated the UV laser up to 6.42 W to study effect of the UV laser power on the printing. The color strength of the printed films was estimated by a spectrophotometer as total color difference (dE).

Results: Particle size, crystal structure, and concentration of TiO_2 in the films did not affect the printing. In the relationship between the irradiated UV laser power and dE, there found an inflection point (1.6 W). When the UV laser power was below 1.6 W, the films were not printed. When it was beyond the point, total color difference increased linearly in proportion with the irradiated laser power.

Conclusions: The color strength of the printing on film was not changed by TiO_2 particle size, crystal structure, and concentration, but could be controlled by regulating the irradiated UV laser power beyond the inflection point.

Keywords: Printing, film-coated tablets, color strength, UV laser, laser energy, titanium dioxide, total color difference (dE), particle size, anatase, rutile

Introduction

Method and identification system to keep track of individual medicines dispensed to patients in a hospital is important to avoid severe health risks. Medicines come in a variety of shapes, sizes, and colors to help distinguish one from the other. However, there is a restriction in keeping good identification characteristics only with the differences in size, shape, color of tablets or capsules so that marking directly on the medicines become more important for identification method. For example, in the case of film-coated tablets, an embossing or a printing has been used for the marking. In the case of capsule, the printing also has been used generally.

The embossing is a method to manufacture coded film-coated tablets by coating the embossed core tablets[1,2]. However, the information for identification is so restricted. Offset printing, ink-jet printing, and pad printing are the popular printing methods to print marks on surface of tablets or capsules using an ink. Since those are easy to be affected by the surface roughness of tablets or capsules, environmental conditions of process room, it is necessary to strictly control viscosity, uniformity, temperature, and drying of the ink to obtain a clear printing during manufacturing. In addition, the printable area is so restricted that the information for identification, for example, number and size of letters are not sufficient yet.

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In the previous papers[3,4], we reported about development, characteristics, and printing mechanism of an ultraviolet (UV) laser-marking device, which imprints on the surface of film-coated tablets by irradiation of UV laser at a wavelength of 355 nm. Titanium dioxide (TiO_2) in the film played an important role for the printing on film-coated tablets by the UV laser. When the UV laser was irradiated to film containing TiO_2, many amounts of black particles, which were agglomerates of grayed oxygen-defected TiO_2 formed by the UV laser irradiation, were formed in irradiated spots of the film and the spots showed their color change from white to gray. The marking would be stable under the condition of 40°C/75%RH for 6 months, or an irradiation of 1200×10^3 l×·h visible-light exposure by xenon lamp. The UV laser irradiation to enteric film-coated tablets did not destroy the film's integrity. The machine makes it possible to print even complicated designs or many letters clearly on film-coated tablets and to keep productivity high. However, factors controlling color strength of the printing on the film were not cleared.

TiO_2 is well known as not only as a popular pharmaceutical excipient[5–7] or as an opacifier in film of film-coated tablets but also a photocatalyst[8–15] for waste material treatment, by a wide range of industries. When TiO_2 is illuminated with UV light of an appropriate wavelength, it generates highly active oxidizing sites that show strong oxidation[16,17]. There has been an increasing interest in environmental applications and photocatalysis, which was studied by many researchers. Surface morphology of the photocatalyst, namely particle size or agglomerate size, is a very important parameter influencing the photocatalytic degradation of chemical compounds[8–11]. Three different crystal structures of TiO_2 exist, rutile, anatase, and brookite and generally rutile and anatase are used in industries. Lee et al., reported that there were not so many differences in photoactivities between anatase and rutile when the particle size was same. But in general, anatase is found to be more photoactive[8,12]. Kaneco et al.[13], reported effects of photocatalyst dosage on degradation of di-*n*-butyl phthalate in aqueous solution. The degradation efficiency increased with increasing amount of TiO_2 and then, the efficiency became nearly flat. The increase in the efficiency seemed to be due to the increase in the total surface area. When TiO_2 was overdosed, a number of active sites on the TiO_2 surface area may be almost constant because of the decreased light penetration and the increased light scattering. The UV light illumination to TiO_2 causes these photocatalytic phenomenons. In the case of the printing on film-coated tablets by the UV laser, the irradiation to TiO_2 also causes the printing on film[3]. However, factors controlling the color strength of printing on film by the UV laser irradiation were not cleared.

In this paper, we examined effects of particle size, crystal structure, or concentration of TiO_2 in film, and irradiated UV laser power on color strength of the printing on film.

Materials and methods

Materials

Characteristics of TiO_2 used in this study were listed in Table 1. All of the TiO_2 samples were kindly gifted by the suppliers. Hydroxypropylmethylcellulose2906 (HPMC) was purchased from Shin-Etsu Chemical Co., Ltd., Tokyo, Japan. Polyethylene glycol 8000 (PEG-8000) was from NOF Corporation, Tokyo, Japan. Talc was from Matsumurasangyo Co., Ltd., Osaka, Japan.

Film preparation[3]

HPMC is one of the most popular pharmaceutical polymers for pharmaceutical film-coated tablets[18–22] so that in this study, formulations listed in Table 2 were used for preparation of films. TiO_2 was dispersed in suspension consisting of HPMC, PEG-8000, talc, and purified water by homogenization for 10 min at 5000 rpm using a Polytron homogenizer (Kinemachika AG., Lucerne, Switzerland). Each of 5 g of the suspensions was deposited on a plastic Petri dish of 11 cm diameter by a dropping pipette after removal of bubbles by stirring the suspension gently and was spread uniformly. After drying in a tray drier at 50°C, films were obtained and were used for experiments.

Table 1. Characteristics of TiO_2 used in this study.

Brand name	KA-30S	KA-10M	NA65	A-HR	TA-300
Supplier	Titan Kogyo Ltd.	Titan Kogyo Ltd.	Toho Titanium Co., Ltd.	Freund Corporation	Fuji Titanium Industry Co., Ltd.
Address	Ube, Japan	Ube, Japan	Chigasaki, Japan	Tokyo, Japan	Osaka, Japan
Crystal structure	Anatase	Anatase	Rutile	Anatase	Anatase
TiO_2 purity (%)	98.1	98.0	99.7	100.3	98.9
BET surface area (m^2/g)	12.20	9.34	8.33	8.68	7.00
BET particle size (nm)	126.1	164.7	170.2	177.2	219.8

The surfaces of the all TiO_2 powder are not modified by any organic or inorganic compounds. The BET surface area was determined using Coulter SA3100 (Beckman Coulter Inc., Fullerton, CA) and the BET particle size was calculated from the BET surface area by following equation assuming non-porous spheres with uniform particle size $d = 6/(\rho \cdot Sw)$ The d, ρ, and Sw are BET particle size, true density, and the BET surface area, respectively. The ρ for anatase or rutile is 3.90 or 4.23 g/cm^3, respectively[25].

UV laser irradiation

A tripled Nd:YVO4 laser (DS20H-355, Photonics Industries International, Inc., NY, USA), producing pulsed laser of up to 8 W peak pulse power by AO-Q switch to 20 kHz, was used for UV laser irradiation. The pulse repetition rate of the laser was 20 kHz. The laser was set to a wavelength 355 nm and diameter of the laser spot was 0.1 mm. The films were irradiated the pulsed UV laser by scanning at the speed of 2000 mm/s parallel with 0.5 mm distance lattice controlled by CAD system.

Total color difference (dE)

Color strength of films was examined by a spectrophotometer (SD5000, Nippon Denshoku Co. Ltd., Tokyo, Japan) using a standard white plate as a reference. This enables examination of the L*a*b* co-ordinate which closely represents human sensitivity to color. Equal distances in this system equal perceived color differences where L* is the lightness variable while a* (green to red) and b* (blue to yellow) are chromaticity co-ordinates. The color strength of the printing films was estimated by a spectrophotometer as total color difference (dE), which is a straight-line distance between co-ordinates of the sample and the standard white plate, and is calculated using Equation 1.

$$dE = [(dL^*)^2 + (da^*)^2 + (db^*)^2]^{1/2} \quad (1)$$

Results and discussion

Effect of TiO_2 particle size or crystal structure on color strength of printing on film

To estimate effects of TiO_2 particle size or crystal structure on color strength of printing on film by the UV laser irradiation, anatase or rutile was used. The characteristics of the TiO_2 used in this study were listed in Table 1. BET particle sizes of the anatase were ranging from 126.1 to 219.8 nm. BET particle size of the rutile was 170.2 nm. Figure 1 shows the relationship between TiO_2 particle size and dE, color strength of printing on film containing 4.0% of TiO_2 by 3.14 W of the UV laser irradiation. When films containing anatase were irradiated the UV laser, the total color difference (dE) of the printed film was constant regardless of the TiO_2 particle sizes.

Photocatalytic activities of TiO_2 are induced by UV light illumination and in general, TiO_2 particle size is an important parameter for the photocatalysis since it directly impacts a specific surface area of a catalyst. With small particle size, number of active surface sites increases[8–11]. Using methylene blue, Xu et al.[10], reported that photocatalytic activity of TiO_2 increased as TiO_2 particle size became smaller, especially when the particle size was less than 30 nm. Almquist and Biswas[11] studied effect of particle size on photo-oxidation rate using TiO_2 with size ranging from 5 to 165 nm, and reported that the apparent photo-oxidation rate of phenol increased as the particle size increased from 5 to 40 nm and decreased as the particle size increased beyond 40 nm. In the case of printing on film by the UV laser, it was found that TiO_2 particle size, which was ranging from 126.1 to 219.8 nm, did not affect color strength of the printing by the UV laser as shown in Figure 1.

Three different crystal structures of TiO_2 exist, rutile, anatase, and brookite. Rutile or anatase, in general, is used in industries. All of the TiO_2 used in this study were anatase except for NA65 obtained from Toho Titanium Co. Ltd., Chigasaki, Japan which was rutile (Table 1). Figure 1 also shows a comparison of dE of the printed films between anatase (closed circles) and rutile (open circles). It was reported that anatase was usually found to be more photoactive[8,12]. On the other hand, Lee et al.[23], reported that there were not so differences in photoactivities between anatase and rutile when the particle size was same. In the case of the printing by the UV laser, it was found that there was no difference in color strength of the printing on film between anatase and rutile.

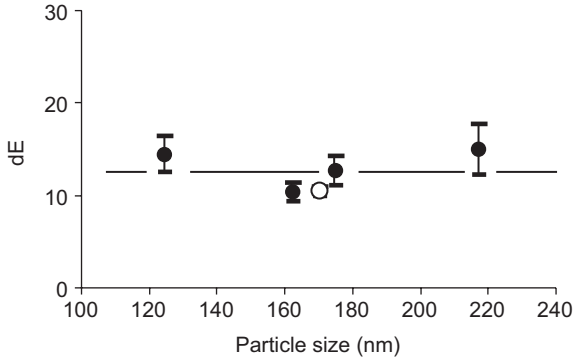

Figure 1. Relationship between TiO_2 particle size and total color difference of printed film (dE). The TiO_2 concentration in the film was 4.0% and the films were irradiated 3.14 W of ultraviolet laser at a wavelength of 355 nm. Closed circles show anatase and open circle shows rutile. Each point represents the mean ± SD ($n = 5$).

Table 2. Formulations of the films used in this study.

TiO_2 concentration (%)	1.0	2.5	4.0	5.9	7.7
TiO_2	0.2 g	0.5 g	0.8 g	1.2 g	1.6 g
HPMC	11.2 g	11.2 g	11.2 g	11.2 g	11.2 g
PEG-8000	2.4 g	2.4 g	2.4 g	2.4 g	2.4 g
Talc	5.6 g	5.6 g	5.6 g	5.6 g	5.6 g
Purified water	180.0 g	180.0 g	180.0 g	180.0 g	180.0 g

The materials were dissolved or suspended in the purified water. Each of the 5 g suspensions was spread with a plastic Petri dish of 11 cm diameter and the films were obtained after drying.

Effect of TiO_2 concentration in film on color strength of printing on film

When the UV laser was irradiated to film containing TiO_2, many amounts of black particles, which were agglomerates of grayed oxygen-defected TiO_2, were formed in irradiated spots and the spots led their color change from white to gray[3]. So, it was assumed that TiO_2 concentration in the film might affect color strength of printing on the film. That is to say that the higher the TiO_2 concentration in film was, the grayer the color strength of the printing might be. Figure 2 shows the relationship between TiO_2 concentration in film and dE of the printed film. The TiO_2 was anatase and the particle size was 164.7 nm. When the films containing TiO_2 concentration ranging from 1.0 to 7.7% were irradiated, whether it was 3.14 W (closed circles) or 5.39 W (open circles) of the UV laser, the total color differences were constant regardless of the TiO_2 concentration which were 10.5 or 22.7, respectively. The color strength of the printing on film by 5.39 W UV laser irradiation was grayer than that by 3.14 W.

As shown in Figure 2, the TiO_2 concentration in film did not affect the color strength of printing on film by the UV laser but the irradiated UV laser energy affected the color strength of printing. Kaneco et al.[13], reported effect of photocatalyst dosage on degradation of di-*n*-butyl phthalate in aqueous solution. The degradation efficiency increased with increasing amount of TiO_2 and then, the efficiency became nearly flat when TiO_2 was overdosed because of the decreased light penetration and the increased light scattering of TiO_2. In the case of the photocatalystic study, UV light was illuminated for a long period so that their consideration that amount of TiO_2 played an important role for the photocatalyst might be reasonable. On the other hand, in the case of printing on film by the UV laser, the UV laser irradiation was performed as 20 ns pulses, very short periods so that the film, of which TiO_2 concentration was more over 1.0%, had sufficient amount of TiO_2 particles dispersed in the film for printing. The TiO_2 concentration ranging from 1.0 to 7.7% in film was not a limiting condition for the UV laser printing but the irradiated UV laser energy might be a limiting condition as shown in Figure 2. It was found that TiO_2 concentration, more over 1.0% in film did not affect color strength of the printing on film by the UV laser.

Effect of UV laser power on color strength of printing on film

Figure 3 shows the relationship between the irradiated UV laser power and dE of the printed film in the case of film containing 4.0% of TiO_2. The TiO_2 was anatase and the particle size was 164.7 nm. Ranging TiO_2 concentration from 1.0 to 7.7% in film, the TiO_2 concentration did not affect color strength of the printing on films by the UV laser as shown in Figure 2 so that effect of the UV laser power on color strength of printing was investigated using the film containing 4.0% of TiO_2. When the irradiated UV laser power was lower than 1.6 W, which was calculated to 0.08×10^{-3} J/pulse by Equation 2, the films were not printed and looked white. Total color difference was constant and was about 2.8.

$$E = \frac{P}{R} \tag{2}$$

where, E, P, and R are output laser energy (J) of each pulse, output power (W) of the laser and pulse repetition rate (Hz) of the pulsed laser, respectively.

When films were irradiated more than 1.6 W UV laser, total color difference of the film increased linearly in proportion with the irradiated UV laser power.

In this study, we found that there existed an inflection point ((A) in Figure 3) in the relationship between dE of the printed film and the irradiated UV laser power and that beyond the inflection point, the dE of the printed film increased linearly in proportion with the irradiated UV laser power. Previously, we reported[3] that amounts of black particles, which were agglomerates of grayed oxygen-defected TiO_2, were formed in the irradiated spots of film when the UV laser was irradiated to the film containing TiO_2 and that the formation of the particles

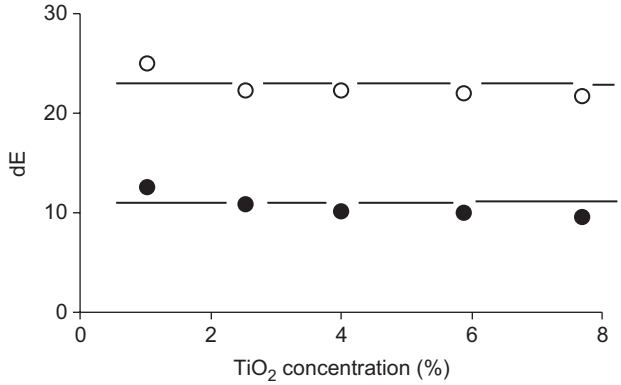

Figure 2. Relationship between TiO_2 concentration in film and total color difference of printed film (dE). Titanium dioxide was anatase, of which particle size was 164.7 nm. The films were irradiated 3.14 W (closed circles) or 5.39 W (open circles) ultraviolet laser. Each point represents the mean ($n = 4$). SD of each point was smaller than the symbol.

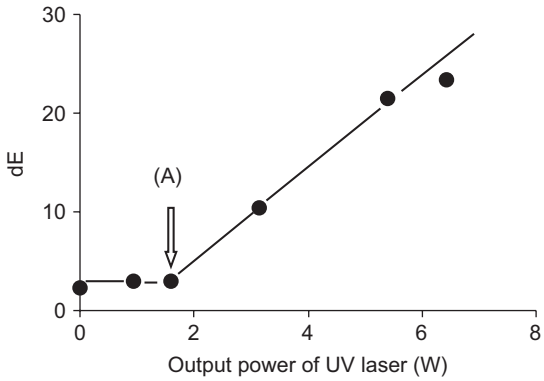

Figure 3. Relationship between ultraviolet laser energy and total color difference of printed film (dE). Titanium dioxide (TiO_2) was anatase, of which particle size was 164.7 nm. TiO_2 concentration in the film was 4.0%. Each point represents the mean ($n = 4$). SD of each point was smaller than the symbol. (A) is an inflection point and is 1.6 W.

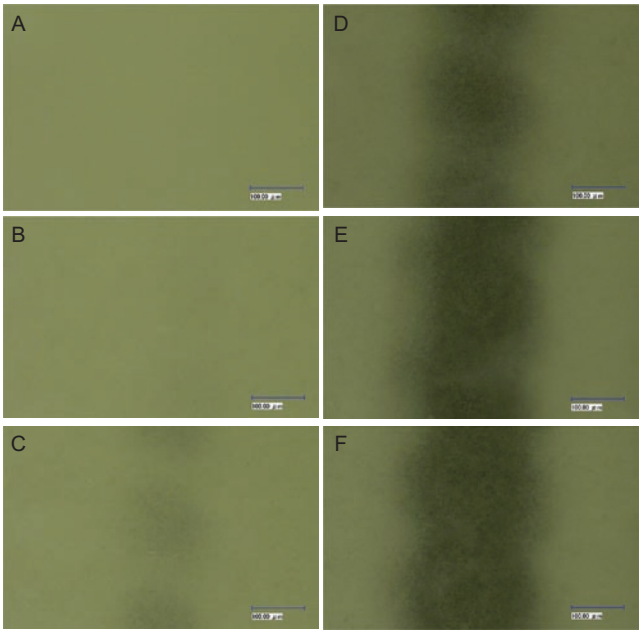

Figure 4. Photographs (×500) of spots irradiated each of ultraviolet (UV) energies by zoom stereo microscope. The irradiated UV laser energy was (A) 0 W, (B) 0.95 W, (C) 1.59 W, (D) 3.14 W, (E) 5.39 W, or (F) 6.42 W, respectively.

was associated with the printing on film. Lee et al.[23,24], reported that a visible change in its color from white to gray was observed when TiO_2 powder was irradiated UV laser and that the initial rate of change of total color difference was related to the laser power and at higher pulsed powers, the color change in TiO_2 was more pronounced. Oxygen deficiency might have occurred to TiO_2 powder, closely related to the irradiated UV laser power. However, to form the agglomeration of the grayed oxygen-defected TiO_2 to the black particles for the printing film, the pulsed UV laser energy more than the inflection point (0.08×10^{-3}J/pulse) might be needed.

Figure 4 shows photographs (×500) of the spots irradiated each level of the UV laser power (0, 0.95, 1.59, 3.14, 5.39, or 6.42 W) by Zoom Stereo Microscope (VHX-900, Keyence Co., Ltd., Osaka, Japan). When the UV laser power was below the inflection point, formation of the black particles was not observed. When the UV laser power was beyond the inflection point, it was found that the higher the irradiated UV laser power became, more number of black particles were formed in the irradiated spots. This shows that the inflection point of the irradiated UV laser energy was the minimum energy required to form the black particles on the film.

In this study, we found that color strength of printing on film by the UV laser could be controlled by regulating the irradiated UV laser energy beyond the inflection point (0.08×10^{-3}J/pulse).

Conclusions

In this study, we examined effects of TiO_2 particle size (ranging from 126.1 to 219.8 nm), crystal structure, or concentration in film and irradiated UV laser power on color strength of printing on film by the UV laser irradiation. Total color difference of the printing was not affected by TiO_2 particle size, crystal structure, and concentration in the film. UV laser power did affect color strength of the printing on film. There was an inflection point in the relationship between dE of the printed film and the irradiated UV laser power. Below the inflection point of the power, films were not printed. On the other hand, beyond the inflection point of the power, total color difference increased linearly in proportion with the irradiated UV laser power.

Color strength of printing by the UV laser was not changed by particle size, crystal structure, and TiO_2 concentration in film, but could be controlled by regulating the irradiated UV laser power beyond the inflection point (0.08×10^{-3}J/pulse).

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Kim S, Mankad A, Sheen P. (1986). The effect of rate of coating suspension on incidence of the bridging of monograms on aqueous film-coated tablets. Drug Dev Ind Pharm, 12: 801–809.
2. Rowe RC, Forse SF. (1980). The effect of film thickness on the incidence of the defect bridging of the intagliations on film-coated tablets. J Pharm Pharmacol, 32:583–584.
3. Kato Y, Nakashima Y, Shino N, Sasaki K, Hosokawa A, Ishihara H. (2010). Studies on the mechanism of printing film-coated tablets containing titanium dioxide in the film by using UV laser irradiation. Drug Dev Ind Pharm, 36:405–412.
4. Sasaki K, Shino N, Nakashima Y, Momoi K, Kato Y. (2009). Development of UV laser-marking machine for film-coated tablets. J Jpn Soc Pharm Mach & Eng, 18:5–14.
5. Bechard SR, Ouraishi O, Kwong E. (1992). Film coating: effect of titanium dioxide concentration and film thickness on the photostability of nifedipine. Int J Pharm, 87:133–139.
6. Kato Y, Sasakura D, Miura T, Nagatomo A, Terada K. (2008). Evaluation of risk and benefit in the application of near-infrared spectroscopy to monitor the granule coating process. Pharm Dev Technol, 13:205–211.
7. Rowe RC. (1995). Knowledge representation in the prediction of the opacity of tablet film coating containing titanium dioxide. Eur J Pharm Biopharm, 41:215–218.
8. Jang HD, Kim S-K, Kim S-J. (2001). Effect of particle size and phase composition of titanium dioxide nanoparticles on the photocatalytic properties. J Nanopart Res, 3:141–147.
9. Koči K, Obalová L, Matějová L, Plachá D, Lacný Z, Jirkovský J, Šolcová O. (2009). Effect of TiO2 particle size on the photocatalytic reduction of CO2. Appl Catal, B:Environ, 89:494–502.

10. Xu N, Shi Z, Fan Y, Dong J, Shi J, Hu MZ-C. (1999). Effect of particle size of TiO2 on photocatalytic degradation of methylene blue in aqueous suspensions. Ind Eng Chem Res, 38:373–379.
11. Almquist CB, Biswas P. (2002). Role of synthesis method and particle size of nanostructured TiO2 on its photoactivity. J Catal, 212:145–156.
12. Okamoto K, Yamamoto Y, Tanaka H, Itaya A. (1985). Heterogeneous photocatalytic decomposition of phenol over TiO2 powder. Bull Chem Soc Jpn, 58:2015–2022.
13. Kaneco S, Katsumata H, Suzuki T, Ohta K. (2006). Titanium dioxide mediated photocatalytic degradation of dibutyl phthalate in aqueous solution-kinetics, mineralization and reaction mechanism. Chem Eng J, 125:59–66.
14. Sasirekha N, Basha SJS, Shanthi K. (2006). Photocatalytic performance of Ru doped anatase mounted on silica for reduction of carbon dioxide. Appl Catal, B:Environ, 62: 169–180.
15. Tseng I-H, Chang W-C, Wu JCS. (2002). Photoreduction of CO2 using sol-gel derived titania and titania-supported copper catalysts. Appl Catal, B:Environ, 37:37–48.
16. Fujishima A, Honda K. (1972). Electrochemical photolysis of water at a semiconductor electrode. Nature, 238:37–38.
17. Inoue T, Fujishima A, Konish S, Honda K. (1979). Photoelectrocatalytic reduction of carbon dioxide in aqueous suspensions of semiconductor powders. Nature, 277:637–638.
18. Rowe RC. (1977). The adhesion of film coatings to tablet surface-the effect of some direct compression excipients and lubricants. J Pharm Pharmacol, 29:723–726.
19. Rowe RC. (1980). The molecular weight and molecular weight distribution of hydroxypropyl methylcellulose used in the film coating of tablets. J Pharm Pharmacol, 32:116–119.
20. Banker G, Peck G, Ja S, Pirakitikulr P, Taylor D. (1981). Evaluation of hydroxypropyl cellulose and hydroxypropyl methyl cellulose as aqueous based film coatings. Drug Dev Ind Pharm, 7:693–716.
21. Hogan JE. (1989). Hydroxypropylmethycellulose sustained release technology. Drug Dev Ind Pharm, 15:975–999.
22. Dahl TC, Calderwood T, Bormeth A, Trimble K, Piepmeier E. (1990). Influence of physicochemical properties of hydroxypropylmethylcellulose on naproxen release from sustained release matrix tablets. J Control Release, 14:1–10.
23. Lee S-K, Robertson PKJ, Mills A, McStay D. (1999). Modification and enhanced photocatalytic activity of TiO2 following exposure to non-linear irradiation sources. J Photochem Photobiol A: Chem, 122:69–71.
24. Lee S-K, Robertson PKJ, Mills A, McStay D, Elliot N, McPhail D. (2003). The alteration of the structural properties and photocatalytic activity of TiO2 following exposure to non-linear irradiation sources. Appl Catal B: Environ, 44:173–184.
25. Budavari S (Ed.). (1996). Titanium dioxide. In: The Merck Index. NJ, USA: Merck & Co., Inc., pp.1617.