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Stability of cyanocobalamin in sugar-coated tablets

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Abstract

The purpose of this study was to clarify the stability of cyanocobalamin (VB_{12}-CN) in sugar-coated tablets containing fursultiamine hydrochloride (TTFD-HCl), riboflavin (VB_2), and pyridoxine hydrochloride (VB_6), and to identify the factors affecting the stability of VB_{12}-CN in these sugar-coated tablets. The stability of VB_{12}-CN was investigated using high-performance liquid chromatography while decomposition was evaluated kinetically. The decomposition of VB_{12}-CN in sugar-coated tablets with high equilibrium relative humidity (more than 60%) under closed conditions showed complex kinetics and followed an Avrami–Erofe'ev equation, which expresses a random nucleation (two-dimensional growth of nuclei) model. We showed that equilibrium relative humidity, the incorporation of VB_2 and VB_6, and sugar coating, are the main factors influencing decomposition and that these factors cause the complex decomposition kinetics.

Keywords: Cyanocobalamin; Stability; Sugar-coated tablets; Equilibrium relative humidity; Riboflavin; Pyridoxine hydrochloride

1. Introduction

Vitamin B combinations are a well-recognized treatment of nerve pain, muscle pain, tired eyes, stiff shoulders, and lumbar pain, and usually consist of a combination of thiamine (VB_1), riboflavin (VB_2), pyridoxine hydrochloride (VB_6), and cyanocobalamin (VB_{12}-CN). Fursultiamine hydrochloride (TTFD-HCl) is a well-known thiamine derivative with a better bioavailability than thiamine in humans, and is therefore often used in preference to thiamine in such combinations. Several dosage forms of such vitamin B combinations are available, such as bottled nutritive drinks, capsules, and tablets. It is often necessary to take vitamin B combinations for long periods and, as patients usually prefer to take sugar-coated tablets, these are often used to increase compliance. Sugar-coated tablets have several advantages over other formulations: masking of the unpleasant odor and taste of vitamins, elegant appearance, and ease of swallowing.

When developing sugar-coated tablets containing a vitamin B combination, it is necessary to consider the stability of the vitamins, in particular the stability of VB_{12}-CN. There have been a number of studies on the stability of VB_{12}-CN, which is known to be incompatible with other vitamins such as ascorbic acid, niacinamide, VB_1, and VB_6 (Harada et al., 1969; Terao et al., 1980; DeRitter, 1982; Toyama Pharmaceutical Research Association, 1992; Ichikawa et al., 2005; The Japanese Pharmacopoeia Fourteenth Edition, 2001). Several different analytical methods for the measurement of VB_{12}-CN content have been described in the literature. While both a colorimetric method and a bioassay method have been used (Harada et al., 1969; Terao et al., 1980; The Japanese Pharmacopoeia Fourteenth Edition, 2001), neither method is capable of discriminating between VB_{12}-CN and its decomposition products. Therefore, a high-performance liquid chromatography (HPLC) method which is able to discriminate between these compounds has been developed. This method has been widely used for measurements of VB_{12}-CN content (Toyama Pharmaceutical Research Association, 1992; Ichikawa et al., 2005). From the viewpoint of separation analysis, the HPLC method is now widely recognized as being more accurate and reliable than other analytical methods. There are, however, few studies on the stability of VB_{12}-CN or on the decomposition kinetics of VB_{12}-CN in solid dosage forms which have used the HPLC method, in spite of the fact that VB_{12}-CN is a well-known vitamin and widely

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used in solid dosage forms in the pharmaceutical field. In particular, there are few stability studies of VB_{12}-CN in sugar-coated tablets using the HPLC method. In the present study, we investigated the stability and decomposition kinetics of VB_{12}-CN in sugar-coated tablets containing TTFD-HCl, VB_2, and VB_6 using the HPLC method, and compared it with the stability of VB_{12}-CN in powders, granules, and plain tablets. In order to clarify the factors affecting the stability of VB_{12}-CN in sugar-coated tablets, we evaluated the stability of VB_{12}-CN in sugar-coated tablets under various conditions of equilibrium relative humidity (ERH), in sugar-coated tablets lacking one of the other vitamins, and in halved sugar-coated tablets.

2. Materials and methods

2.1. Materials

The following vitamins were used in the study: cyanocobalamin (VB_{12}-CN, Aventis Pharma S.A., France), fursultiamine hydrochloride (TTFD-HCl, Takeda Pharmaceutical Company Limited, Japan), riboflavin (VB_2, Takeda Pharmaceutical Company Limited, Japan), and pyridoxine hydrochloride (VB_6, Takeda Pharmaceutical Company Limited, Japan). Lactose (Meggle GmbH, Germany) was used as a diluent. Corn starch (Nihon Cornstarch Co., Japan) was used as both a diluent and a disintegrant. Hydroxypropylcellulose (HPC-L, Nippon Soda Co., Ltd., Japan) was used as a binder. Magnesium stearate (Sakai Chemical Industrial Co., Ltd., Japan) was used as a lubricant. Sucrose (Ensuiko Sugar Refining Co., Japan), talc (Matsumura Sangyo Co., Japan), titanium dioxide (Ishihara Sangyo Kaisha Ltd., Japan), and powdered acacia (San-ei Yakuhin Boueki Co., Japan) were used for sugar coating. The above vitamins and excipients were in compliance with The Japanese Pharmacopoeia Fourteenth Edition (2001). All other chemicals were of reagent grade.

2.2. Manufacture of granules and plain tablets

TTFD-HCl, VB_2, VB_6, lactose, and corn starch were granulated with 0.12% VB_{12}-CN aqueous solution and 6% HPC-L aqueous solution using a fluidized-bed granulator (FD-3S, Powrex Co., Japan). Loaded weight in the fluidized-bed granulator was 1092 g, inlet air temperature 70 °C, spray air pressure 0.1 MPa, and spray feed rate 8–10 g/min. After granulation, the granules were milled using a screen mill (Power Mill, Showa Kagaku Kikai Kosakusho Co., Ltd., Japan) with 1.5 mm screen size. The milled granules were mixed manually with corn starch and magnesium stearate in a plastic bag. Lubricated granules were compressed using a rotary compression machine (Correct 19 K, Kikusui Seisakusho Ltd., Japan) with 8-mm diameter convex punches (radius 6.5 mm). Tablet weight was 170 mg, compression pressure 6–8 kN/punch, and rotation speed 20 rpm.

Each plain tablet contained 0.01 mg VB_{12}-CN, 54.58 mg TTFD-HCl, 4.95 mg VB_2, 5.0 mg VB_6, 68.46 mg lactose, 31.5 mg corn starch, 5.0 mg HPC-L and 0.5 mg magnesium stearate. When tablets lacking one of the vitamins were manufactured, the quantity of lactose was adjusted to maintain a constant tablet weight of 170 mg as a plain tablet.

2.3. Sugar coating

Sugar coating was performed manually on 5500 plain tablets in a 12-in. onion pan (Kikusui Seisakusyo Ltd., Japan) using a dusting method (Ohmori et al., 2004a,b). The dusting method can be divided into four steps: (1) subcoating, (2) smoothing, (3) colouring, and (4) polishing.

(1) The subcoating is applied to round the edges and build up the tablet size. The subcoating step consists of alternately applying a sugar-coating suspension to the tablets followed by dusting with powders and drying. Firstly, the sugar-coating suspension is added to the core tablets. Secondly, the tablets are stirred by hand to distribute the suspension. Thirdly, the dusting powder is applied until all the tablets are coated and tumble freely. Finally, the tablets are dried by hot air at 60 °C and the excess dusting powder is removed through the exhaust. The sugar-coating suspension formulation is 45.0% (w/w) sucrose, 25.4% (w/w) talc, 2.8% (w/w) titanium dioxide, 4.3% (w/w) powdered acacia, and 22.5% (w/w) purified water. The dusting powder formulation is 98% (w/w) talc and 2% (w/w) powdered acacia. The weight of a final subcoated tablet was 252.3 mg.
(2) The smoothing step is to smoothe out the tablet surface prior to application of the colouring, and consists of alternately applying the sugar-coating suspension to the tablets and drying. The drying temperature was 60 °C. The weight of a final smoothed tablet was 294.5 mg.
(3) In the colouring step, the desired colour is imparted to the tablets by alternately applying a colouring syrup and drying. The colouring syrup formulation is 66.55% (w/w) sucrose, 0.12% (w/w) VB_2, and 33.33% (w/w) purified water. The drying temperature is initially 55 °C, and is gradually reduced to 25 °C. The weight of a final coloured tablet was 320.0 mg.
(4) The polishing step is to achieve a final gloss, and involves application of a mixture of waxes (beeswax and carnauba wax) to the tablets in a polishing pan.

2.4. Moisture content

2.4.1. LOD of powders and granules

Moisture contents of VB_{12}-CN powders and granules containing VB_{12}-CN, TTFD-HCl, VB_2, and VB_6 were measured by loss on drying (LOD). The samples (1 g) were dried at 60 °C for 5 h under the vacuum condition (0.53 ± 0.13 kPa) using equipment (TD-40G, Toyama Sangyo Co., Ltd., Japan).

2.4.2. ERH of tablets

The ERH is water activity expressed as a percentage (Heidemann and Jarosz, 1991). This was used as a measure of the moisture content of the tablets and was measured using a water activity analyzer (Hygroskop DT, Rotronic, Switzerland). About 1 g of crushed tablets was used for measurement.

2.5. Stability test of VB_{12}-CN

2.5.1. Storage conditions

2.5.1.1. Open conditions. The powders or granules were put into uncapped glass bottles which were stored in desiccators containing various saturated solutions at 40 °C. Humidity conditions depended on the saturated solution used. In other words, under open conditions, the powders or granules in the glass bottle were exposed to both moisture and heat.

2.5.1.2. Closed conditions. The tablets were put into a glass bottle which was then sealed with a metal cap. The closed bottle was stored in the oven at 40 or 60 °C. In other words, under closed conditions, the tablets in the glass bottle were exposed to only heat.

In the storage stability experiments, VB_{12}-CN powders, and granules (after milling) containing VB_{12}-CN, TTFD-HCl, VB_2, and VB_6 were stored in open glass bottles placed within desiccators at 40 °C/6%RH, 40 °C/57%RH, and 40 °C/75%RH (open conditions). Plain tablets containing VB_{12}-CN, TTFD-HCl, VB_2, and VB_6 were moisturized in desiccators using various saturated solutions at 25 °C (open conditions) and then stored in glass bottles at 40 °C under closed conditions. Sugar-coated tablets, whose ERH was controlled during the process of sugar coating, were stored in glass bottles at 40 °C under closed conditions. In the experiment investigating the influence of sugar coating, plain tablets, sugar-coated tablets, and halved sugar-coated tablets were stored at 60 °C under closed conditions.

2.5.2. Measurements of VB_{12}-CN content

When measuring the VB_{12}-CN content of powders and granules, a sample of powders or granules corresponding to the weight of five tablets was accurately weighed. The sample was then dissolved in 25 mL diluent methanol (10%). The sample suspension was shaken for 30 min in a shaker (Recipro shaker SR-2w, Taitec Co., Ltd., Japan) and centrifuged for 10 min at 10,000 rpm (O5P-21 centrifuge, Hitachi, Japan). The clear supernatant liquid was used for the measurement of VB_{12}-CN content. When measuring the VB_{12}-CN content of tablets, 10 tablets were crushed using a mill (8000 SPEX CertiPrep Mixer/Mill, SPEX CertiPrep Inc., USA). The same procedure as described above was then used to prepare the samples.

Measurements were performed using an HPLC method with a system equipped with a controller (600, Waters, USA) and an ultraviolet-visible light detector (SPD-6AV, Shimadzu, Japan). HPLC conditions were as follows: column, YMC-Pack ODS-A-312 (YMC Co., Ltd., Japan); column temperature, 25 °C; detection wavelength, 550 nm; mobile phase, 0.05 mol/L ammonium dihydrogenphosphate aqueous solution (pH 3.5):methanol (3:1); flow rate, 1.0 mL/min; injection volume, 100 μL.

2.6. Measurement of pH in the suspensions

The pH of suspensions of TTFD-HCl (10 g in 10 mL purified water), VB_6 (10 g in 10 mL purified water), granules (1 g in 10 mL purified water), and crushed sugar-coated tablets (one tablet in 10 mL purified water) was measured using a pH meter (F-16, Horiba, Japan).

3. Results and discussion

3.1. Stability of VB_{12}-CN in powders and granules

It is well known that vitamins are susceptible to moisture. Therefore, we investigated the stability of VB_{12}-CN in powders, and in granules containing TTFD-HCl, VB_2, and VB_6 under various conditions of humidity. Fig. 1(a) shows the stability of VB_{12}-CN in powders. VB_{12}-CN was stable in powders stored at 40 °C/6%RH and 40 °C/57%RH but unstable in powders stored at 40 °C/75%RH. The stability of VB_{12}-CN in powders is therefore susceptible to moisture. Fig. 2(a) shows the stability of VB_{12}-CN in granules. VB_{12}-CN was stable in granules stored at 40 °C/6%RH, but unstable in granules stored at 40 °C/57%RH or 40 °C/75%RH. The stability of VB_{12}-CN in granules was more susceptible to moisture than in powders.

In powders and granules stored under conditions of high humidity, decomposition followed neither zero-order nor first-order kinetics. A zero-order equation can be expressed as shown in the following equation:

$$(1 - X) = K_0 t + 1 \quad (1)$$

where X is decomposition rate, t is time, and K_0 is a zero-order rate constant. A first-order equation can be expressed as shown in the following equation:

$$\ln(1 - X) = K_1 t \quad (2)$$

where X is decomposition rate, t is time, and K_1 is a first-order rate constant.

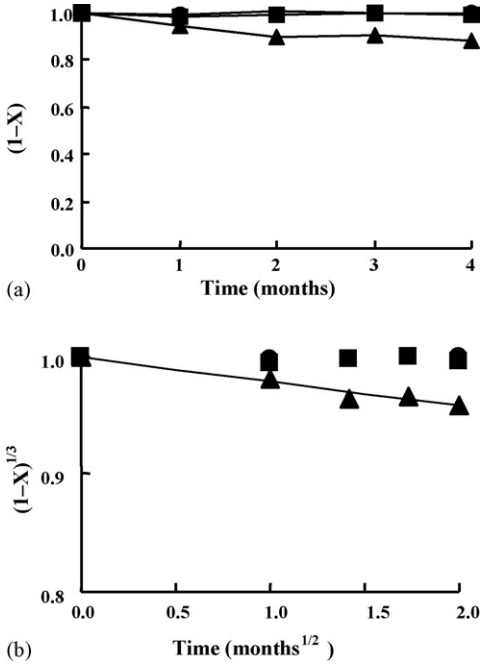

Fig. 1. Stability of VB_{12}-CN in powders. (X) Decomposition rate. (a) Zero-order plots; (b) Jander plots. (●) 40 °C/6%RH; (■) 40 °C/57%RH; (▲) 40 °C/75%RH.

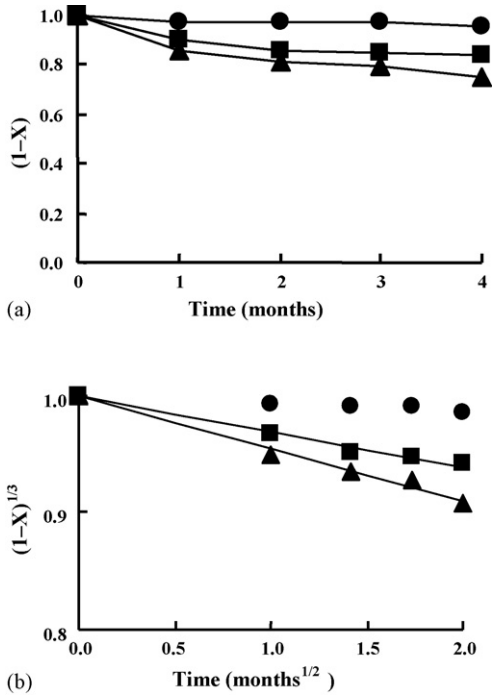

Fig. 2. Stability of VB_{12}-CN in granules containing TTFD-HCl, VB_2, and VB_6. (X) Decomposition rate. (a) Zero-order plots; (b) Jander plots. (●) 40 °C/6%RH; (■) 40 °C/57%RH; (▲) 40 °C/75%RH.

As decomposition followed neither zero-order nor first-order equations, a Jander equation was applied to the decomposition kinetics (Horioka et al., 1974; Okazaki et al., 1989, 1990). Fig. 1(b) and Fig. 2(b) show Jander plots of the stability of VB_{12}-CN in the powders and granules. The decomposition in powders stored at 40 °C/75%RH and in granules stored at 40 °C/57%RH and 40 °C/75%RH was shown to follow the Jander equation, which can be expressed as shown in the following equation:

$$(1 - X)^{1/3} = K_J t^{1/2} + 1 \quad (3)$$

where X is decomposition rate, t is time, and K_J is a Jander rate constant. In this case, the powder and the granules were kept under dry conditions before the experiment. During the experiment, the surrounding moisture reacted with the interfaces of the powders and granules, and VB_{12}-CN decomposed. The decomposition fitted neither the zero-order equation, which expresses a drug-concentration-independent constant reaction model, nor the first-order equation, which expresses a drug-concentration-dependent constant reaction model. The decomposition did fit the Jander equation, which expresses a three-dimensional diffusion model. Rate constants and R^2 values in powders and granules are listed in Table 1. The rate constant for the granules was larger than that for the powders. Moisture contents of the powders stored at 40 °C/6%RH, 40 °C/57%RH, and 40 °C/75%RH were 3.9%, 12.8%, and 15.5%, respectively. Moisture contents of the granules stored at 40 °C/6%RH, 40 °C/57%RH, and 40 °C/75%RH were 2.2%, 3.5%, and 4.1%, respectively. Although the differences in the decomposition rate between in the granules stored at different humidity conditions were due to the differences in moisture contents, the differences in decomposition rate between in the powders and in the granules stored at the same humidity conditions were not due to the differences in moisture contents. The stability of VB_{12}-CN in the granules could be affected by the other vitamins such as TTFD-HCl, VB_2, and VB_6. Furthermore, during the granulation process, VB_{12}-CN aqueous solution was adhered onto the surface of the ingredients, resulting in the dramatic increase of the surface area in drying process. The increase of the surface area of VB_{12}-CN in the granules could contribute to the larger rate constant.

Table 1
Rate constants and R^2 values in powders and granules

	Rate constant	R^2
Powders at 40 °C/75%RH		
Zero-order	−0.03	0.7968
First-order	−0.04	0.8092
Jander	−0.02	0.9553
Granules at 40 °C/75%RH		
Zero-order	−0.07	0.7813
First-order	−0.03	0.8243
Jander	−0.05	0.9886
Granules at 40 °C/57%RH		
Zero-order	−0.05	0.7194
First-order	−0.02	0.7450
Jander	−0.03	0.9780

3.2. Stability of VB_{12}-CN in plain tablets

Moisture in solid dosage forms is generally classified as mobile or immobile water. The measurement of mobile water is expressed by ERH. Much research has been carried out on the relationship between stability and ERH (Heidemann and Jarosz, 1991; Ohmori et al., 2004c), and it is now well recognised that ERH is correlated with the stability of moisture-sensitive drugs. Fig. 3 shows the stability of VB_{12}-CN in plain tablets containing TTFD-HCl, VB_2, and VB_6 with different ERH. VB_{12}-CN in plain tablets with a high ERH (65%) was less stable than in tablets with a low ERH (44%). The stability of VB_{12}-CN in plain tablets was also susceptible to moisture. Although decomposition in powders and granules followed the Jander equation, decomposition in the plain tablets followed the zero-order equation. The difference in decomposition kinetics may be due to

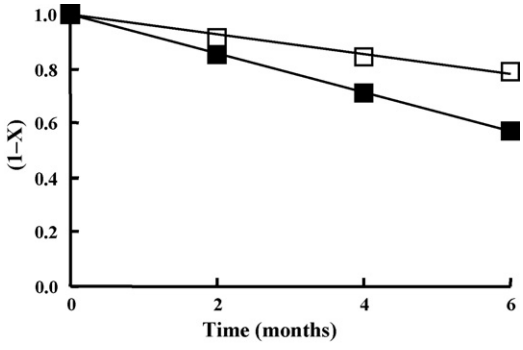

Fig. 3. Stability of VB_{12}-CN in plain tablets containing TTFD-HCl, VB_2, and VB_6 at 40 °C. (X) Decomposition rate. (□) ERH 44%; (■) ERH 65%.

the different storage conditions. While the storage conditions for the powders and granules were open, storage conditions for the plain tablets were closed. Furthermore, the storage of plain tablets was started after controlling their ERH in desiccators using saturated solutions at 25 °C. In other words, the moisture conditions affecting the decomposition were different. Under open conditions, surrounding moisture is able to attack VB_{12}-CN and the decomposition reaction sites would therefore be these interfaces. Under closed conditions, moisture in the plain tablets, which can attack VB_{12}-CN, would be more uniform inside the tablets compared with that in the granules because of the process of controlling ERH. The decomposition reaction sites would therefore be unlimited. Therefore, the decomposition of VB_{12}-CN in plain tablets under closed conditions followed the zero-order equation.

3.3. Stability of VB_{12}-CN in sugar-coated tablets

Fig. 4(a) shows the stability of VB_{12}-CN in sugar-coated tablets containing TTFD-HCl, VB_2, and VB_6. The decomposition of VB_{12}-CN in sugar-coated tablets with low ERH (39% and 41%) followed the zero-order equation. The decomposition kinetics were the same as those for plain tablets. In general, the ERH of sugar-coated tablets is more than 60% because of the dusting method used, which is known as the intermittent spray method. It is more difficult to dry the tablets using this method than using the film-coating method (a continuous spray mist method) as the sugar-coating makes it difficult to remove moisture from inside the tablets during the drying process (Ohmori et al., 2004b, c). The stability of VB_{12}-CN in the sugar-coated tablets with high ERH (>60%) was inferior to that in the sugar-coated tablets with low ERH. The decomposition kinetics of VB_{12}-CN in the sugar-coated tablets with high ERH was evaluated kinetically and found not to follow any of the equations mentioned (i.e., neither zero-order, first-order, nor Jander equations). Instead, the decomposition showed complex kinetics, which could be due to a complex decomposition mechanism such as occurs when the decomposition products themselves accelerate the decomposition. In this case, a Prout–Tompkins equation is used, which can be expressed as shown in the following equation:

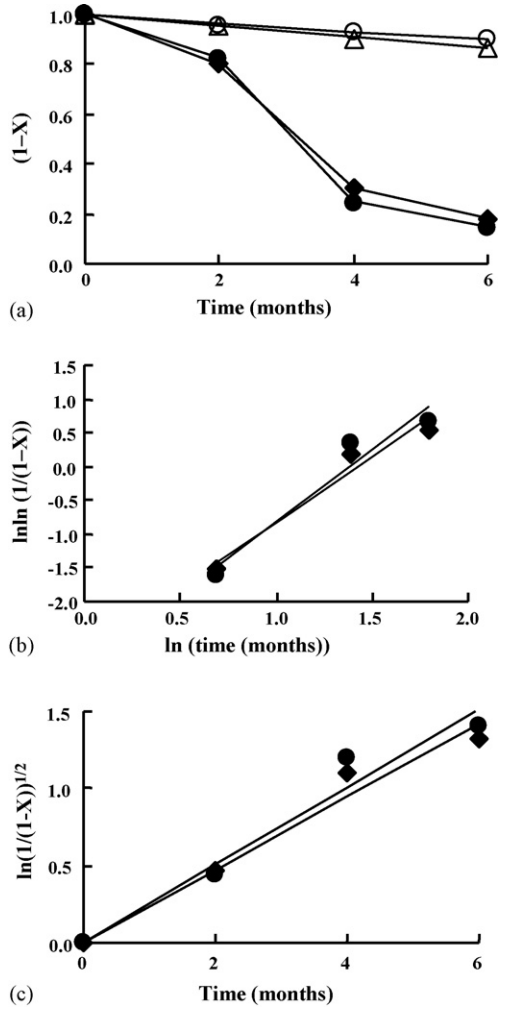

Fig. 4. Stability of VB_{12}-CN in sugar-coated tablets containing TTFD-HCl, VB_2, and VB_6 at 40 °C. (X) decomposition rate. (a) Zero-order plots; (b) Weibull plots; (c) Avrami–Erofe'ev plots. (◯) ERH 39%; (△) ERH 41%; (◆) ERH 65%; (●) ERH 68%.

$$\ln\left(\frac{X}{1-X}\right) = K_{pt}t + C \tag{4}$$

where X is decomposition rate, t is time, K_{pt} is a Prout–Tompkins rate constant, and C is a constant. For example, Carstensen and Attarchi (1988) reported that the decomposition of aspirin in the solid state, in the presence of limited amounts of moisture, followed the Prout–Tompkins equation. However, in the present study, the decomposition of VB_{12}-CN in the sugar-coated tablets did not follow the Prout–Tompkins equation. Yoshioka (1995), when considering that some drugs in solid dosage forms show complex decomposition kinetics, which are not described by any theoretical kinetic equations, suggested that, when it is impossible to apply theoretical decomposition kinetics equations to the decomposition of the drug, the Weibull equation is empirically applicable to the decomposition. Both Okusa (1975) and Seki et al. (1980) have applied the Weibull equation to the decomposition of drugs. Accordingly, we empirically applied the Weibull equation to the decomposition curves. As shown in Fig. 4 (b), the decomposition of VB_{12}-CN in the sugar-coated tablets with high ERH followed the Weibull equation, which can be expressed as shown in the following equation:

$$\ln\ln\left(\frac{1}{1-X}\right) = \ln k + m\ \ln t \tag{5}$$

where X is decomposition rate, t is time, k and m are parameters. The R^2 values were 0.9575 (ERH 65%) and 0.9415 (ERH 68%).

Although the Weibull equation is empirical, kinetic equations can be predicted by using the shape parameter, m, in Eq. (5). The parameter, m, on the decomposition in the sugar-coated tablets were 1.95 (ERH 65%) and 2.17 (ERH 68%). According to the method of Hancock–Sharp (Table 2), an Avrami–Erofe'ev equation would be applicable (Hancock and Sharp, 1972; Umeda et al., 1985). As shown in Fig. 4(c), the decomposition of VB_{12}-CN in the sugar-coated tablets with high ERH followed the

Table 2
Values of m for solid-state decompositions (compiled from Hancock and Sharp, 1972; Umeda et al., 1985)

m	Equation	Mechanism
0.54	$1-(1-X)^{1/3}=kt^{1/2}$ (Jander equation)	Three-dimensional diffusion
0.57	$(1-X)\ln(1-X)+X=kt$	Two-dimensional diffusion
0.57	$(1-2X/3)-(1-X)^{2/3}=kt$ (Ginstling–Brounshtein equation)	Three-dimensional diffusion
0.62	$X^2=kt$	One-dimensional diffusion
1.00	$\ln(1/(1-X))=kt$	Random nucleation (one nucleus on each particle)
1.07	$1-(1-X)^{1/3}=kt$	Phase boundary reaction (spherical symmetry)
1.11	$1-(1-X)^{1/2}=kt$	Phase boundary reaction (cylindrical symmetry)
1.24	$X=kt$	Zero-order mechanism
2.00	$\ln(1/(1-X))^{1/2}=kt$ (Avrami–Erofe'ev equation)	Random nucleation (two-dimensional growth of nuclei)
3.00	$\ln(1/(1-X))^{1/3}=kt$ (Avrami–Erofe'ev equation)	Random nucleation (three-dimensional growth of nuclei)

Avrami–Erofe'ev equation, which can be expressed as shown in the following equation:

$$\ln\left(\frac{1}{1-X}\right)^{1/2}=K_{ae}t \quad (6)$$

where X is decomposition rate, t is time, K_{ae} is an Avrami–Erofe'ev rate constant. The decomposition mechanism of VB_{12}-CN in the sugar-coated tablets with high ERH may be a random nucleation (two-dimensional growth of nuclei). Rate constants and R^2 values in plain and sugar-coated tablets are listed in Table 3.

In order to clarify the factors affecting the stability of VB_{12}-CN in sugar-coated tablets, we investigated the influences of TTFD-HCl, VB_2, and VB_6 on the stability of VB_{12}-CN in sugar-coated tablets (Fig. 5). The stability of VB_{12}-CN in sugar-coated tablets without VB_2 or VB_6, whose ERH is high (67% or 68%), was superior to that in sugar-coated tablets containing VB_2 and VB_6. The decomposition of VB_{12}-CN in sugar-coated tablets without VB_2 or VB_6 followed the zero-order equation. We demonstrated that both VB_2 and VB_6 reduced the stability of VB_{12}-CN and changed the decomposition kinetics of VB_{12}-CN in sugar-coated tablets, with VB_6 having the greater effect. On the other hand, the stability of VB_{12}-CN in sugar-coated tablets without TTFD-HCl was inferior to that in sugar-coated tablets containing TTFD-HCl. TTFD-HCl therefore improved the stability of VB_{12}-CN in sugar-coated tablets. Although VB_{12}-CN is known to be incompatible with thiamine, the precise stabilization mechanism of TTFD-HCl has not been determined in this study. The difference between thiamine and TTFD-HCl is tetrahydrofurfuryl group. Tetrahydrofurfuryl group could contribute to the stabilization of VB_{12}-CN in a measure.

Table 3
Rate constants and R^2 values in plain and sugar-coated tablets

	Rate constant	R^2
Plain tablets (ERH 65%) at 40 °C		
Zero-order	−0.07	0.9996
Plain tablets (ERH 44%) at 40 °C		
Zero-order	−0.04	0.9887
Sugar-coated tablets (ERH 68%) at 40 °C		
Zero-order	−0.15	0.9238
First-order	−0.32	0.9195
Jander	−0.17	0.7779
Prout–Tompkins	0.83	0.8921
Avrami–Erofe'ev	0.25	0.9606
Sugar-coated tablets (ERH 65%) at 40 °C		
Zero-order	−0.15	0.9456
First-order	−0.28	0.9368
Jander	−0.15	0.7995
Prout–Tompkins	0.74	0.9144
Avrami–Erofe'ev	0.24	0.9693
Sugar-coated tablets (ERH 41%) at 40 °C		
Zero-order	−0.02	0.9915
Sugar-coated tablets (ERH 39%) at 40 °C		
Zero-order	−0.02	0.9710
Sugar-coated tablets (without VB_2) (ERH 67%) at 40 °C		
Zero-order	−0.05	0.9799
Sugar-coated tablets (without VB_6) (ERH 68%) at 40 °C		
Zero-order	−0.03	0.9975

pH is one of the most common factors influencing stability. In aqueous solutions VB_{12}-CN is stable between pH 4.0 and pH 7.0, while it is unstable in acidic solutions. Therefore, we measured the pH of suspensions of TTFD-HCl, VB_6, the granules, and crushed sugar-coated tablets and found them to be 2.3, 2.2, 3.1, and 4.1, respectively. Although TTFD-HCl stabilized VB_{12}-CN and VB_6 destabilized VB_{12}-CN, there was no appreciable difference between the pHs of the suspensions of TTFD-HCl and VB_6. In addition, although VB_{12}-CN in the sugar-coated tablets was unstable, the pH of the suspension of

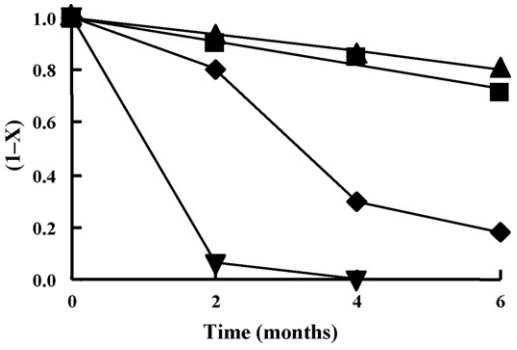

Fig. 5. Influences of TTFD-HCl, VB_2, and VB_6 on stability of VB_{12}-CN in sugar-coated tablets at 40 °C. (X) Decomposition rate. (▲) Sugar-coated tablets without VB_6 (ERH 68%); (■) sugar-coated tablets without VB_2 (ERH 67%); (♦) sugar-coated tablets containing TTFD-HCl, VB_2, and VB_6 (ERH 65%); (▼) sugar-coated tablets without TTFD-HCl (ERH 70%).

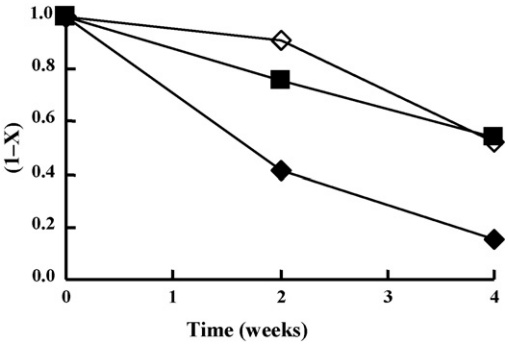

Fig. 6. Influence of sugar coating on stability of VB_{12}-CN in tablets containing TTFD-HCl, VB_2, and VB_6 at 60 °C. (X) Decomposition rate. (■) Plain tablets (ERH 65%); (♦) sugar-coated tablets (ERH 65%); (◊) halved sugar-coated tablets (ERH 65%).

the crushed sugar-coated tablets was in the stable region (pH 4.0–7.0). This showed that the different influences of TTFD-HCl, VB_2, and VB_6 on the stability of VB_{12}-CN were not due to pH.

Fig. 6 shows the influence of sugar coating on the stability of VB_{12}-CN in tablets. Although the ERH levels of the tablets were same, the stability of VB_{12}-CN in the sugar-coated tablets was inferior to that in the plain tablets. Furthermore, although the ingredients and ERH levels in the tablets were the same, the stability of VB_{12}-CN in the halved sugar-coated tablets was superior to that in whole sugar-coated tablets. This suggests that while the ingredients of the sugar-coating layer did not significantly reduce the stability of VB_{12}-CN in the tablets, the sugar-coating layer itself did. Budavari et al. (2001) reported that film-coated tablets containing folic acid, VB_6, and VB_{12}-CN showed improved stability of VB_{12}-CN compared with uncoated tablets. In the present study, sugar coating did not improve the stability of VB_{12}-CN. The difference between sugar-coating and film-coating is the structure of the coating layer. Sugar-coating is a denser coating layer than film-coating, and shows a superior masking ability for unpleasant odors (Ohmori et al., 2004c, 2005), being a gas-tight layer.

Jacob et al. (1968) reported that the content of VB_{12}-CN in film-coated multivitamin tablets was reduced after exposure to methanol vapor for 1 month at room temperature. They postulated that methanol vapor causes decomposition of either ascorbic acid or VB_1, or both, the decomposition products of which in turn influence the stability of VB_{12}-CN. Harada et al. (1969) reported that formic acid, which arises from VB_1 decomposition, decomposed VB_{12}-CN, while Terao et al. (1980) reported that sodium bisulfite decomposed VB_{12}-CN. DeRitter (1982) wrote that VB_{12}-CN is decomposed by the decomposition products of ascorbic acid, the combination of ascorbic acid-Cu^{2+} or dehydroascorbic acid-Cu^{2+}, the 4-methyl-5-(β-hydroxyethyl) thiazole formed by cleavage of thiamine, or the combination of thiamine and niacinamide. Several compounds, in particular the decomposition products of vitamins, have been shown to decompose VB_{12}-CN, but unfortunately they have not been fully identified. Our results suggest that the gases generated inside the sugar-coated tablets containing other vitamins, decompose VB_{12}-CN. We therefore propose the following decomposition mechanism: the combination of sufficient quantities of moisture, VB_2, and VB_6, generates gases in the sugar-coated tablets during storage. Due to the gas-tight coating layer, these gases cannot escape and are free to attack the VB_{12}-CN in the tablets, causing decomposition. This proposed mechanism would account for the complex decomposition kinetics. Acetic acid, formic acid, furfural, and furfur alcohol can all be detected in sugar-coated tablets after storage at 40 °C for 8 months. Unfortunately, the gaseous components related to decomposition were not fully identified in this study. As a result of these findings, we propose that separating VB_{12}-CN from VB_6 and reducing the ERH in the tablets would stabilize VB_{12}-CN in sugar-coated tablets.

4. Conclusions

The decomposition kinetics of VB_{12}-CN in sugar-coated tablets containing TTFD-HCl, VB_2, and VB_6 with high ERH (more than 60%) under closed conditions was different from that in powders kept under open conditions, granules kept under open conditions, plain tablets kept under closed conditions, sugar-coated tablets with low ERH (ca. 40%) kept under closed conditions, and sugar-coated tablets without VB_2 or VB_6 kept under closed conditions. The decomposition kinetics of VB_{12}-CN in powders and granules kept under open conditions followed the Jander equation, which expresses a three-dimensional diffusion model. The decomposition kinetics of VB_{12}-CN in plain tablets, in sugar-coated tablets with low ERH (ca. 40%), and in sugar-coated tablets without VB_2 or VB_6 kept under closed conditions, followed the zero-order equation, which expresses a drug-concentration-independent constant reaction model. The decomposition kinetics of VB_{12}-CN in sugar-coated tablets with high ERH (more than 60%) kept under closed conditions showed complex kinetics and followed the Avrami–Erofe'ev equation, which expresses a random nucleation (two-dimensional growth of nuclei) model. We demonstrated that ERH, the presence of other vitamins (VB_2 and VB_6), and sugar-coating, reduced the stability of VB_{12}-CN and changed the decomposition kinetics of VB_{12}-CN in sugar-coated tablets from a zero-order equation to a complex kinetics equation, the Avrami–Erofe'ev equation.

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