Studies on Dissolution Tests of Soft Gelatin Capsules. V.[1] Rotating Dialysis Cell Method[2]

Masato TAKAHASHI,* Mayumi MOCHIZUKI, Kazumi WADA, Tomoka ITOH, Mihoko OHTA, and Masahiro GOTO

Research & Development Division, Toyo Capsule Co., Ltd., 560, Nakazato Higashi-cho, Fujinomiya-shi, Shizuoka 418, Japan. Received January 31, 1994; accepted April 26, 1994

A dissolution test was performed on a soft-gelatin capsule containing tocopherol nicotinate (TN) diluted with vegetable oil. The test methods were the paddle (PD) method described in the Japanese Pharmacopoeia (JP) and a rotating dialysis cell (RDC) method by which the sample is disintegrated inside a rotating cell covered with a filter and the contents are expelled from the cell into a solution outside.

TN is practically insoluble. In the aqueous test solutions (pH 1.2 and 6.8), low dissolution rates were obtained by both the PD and RDC methods. However, we obtained a high dissolution rate by adding *n*-octanol, which has often been used for the measurement of drug partition coefficient, as a modification of the RDC method which has many factors controlling the drug dissolution. By examining the changes in the blood concentrations of TN after administration of the TN soft-gelatin capsule to humans, we established a relationship between the *in vivo*/*in vitro* findings.

Keywords soft-gelatin capsule; dissolution test; tocopherol nicotinate; rotating dialysis cell method; human; soybean oil

The dissolution test has been widely used in areas of quality control and research and development, and the methodology has recently been much discussed.[3] The test materials are mostly powdered preparations and since they are easily dispersed or dissolved in test solutions such as buffers, the dissolution test can be made by the paddle (PD) method or the rotating basket (RB) method described in the Japanese Pharmacopoeia (JP).

The results of dissolution tests on soft-gelatin capsules (SC) have not been satisfactory because their contents are generally oily. Therefore, we classified the SC according to the base used and determined the most appropriate method for testing the dissolution pattern for each drug group. We previously evaluated the correlation between the *in vitro* and *in vivo* findings from dissolution tests for SC with oily semisolid matrix bases,[4] those with mixtures of oily semisolid matrix bases and water soluble bases,[5] and those with water soluble bases.[1]

In the present study, we selected a fundamental SC composed of an oily drug and a base as the test material. We performed the dissolution tests on a SC containing tocopherol nicotinate (TN) which has often been used as a microcirculation system activator by the PD and rotating dialysis cell (RDC) methods [also called Pharma Test Suppositorium Wirkstoff Freigabe (PTSW) type or Dibbern method],[6] and evaluated the correlation between the *in vivo* findings after oral administration to humans and the *in vitro* findings.

Experimental

Materials and Reagents An oval SC containing 200 mg of *dl*-α-TN, a BASF product, per capsule (TN-SC) was prepared by the rotary die method[7] (Table I). Japan industrial standard guarantee (JIS-GR) reagents or those for liquid chromatography were used.

Apparatus A dissolution test apparatus (NTR-VS3) made by Toyama Sangyo Co., Ltd., and equipped with an RDC apparatus made by Pharmatest Co., Ltd. was used.

Dissolution Test For each capsule of TN-SC, the test was performed according to the following method and the dissolved amounts of TN were measured 15, 30, 60, 120, 180 and 240 min later.

PD Method: The PD method described in the JP was used.

As test solutions, 1000 ml each of the deaerated 1st (pH 1.2) and 2nd (pH 6.8) fluids described in the disintegration test in the JP were used. The temperature was kept at 37±0.5 °C. The paddle rotation rate was 100 rpm. The rate of dissolution of TN dissolved in the solutions at each point was measured by filtration of 5 ml through a membrane filter with a pore size of 0.45 μm, and the amounts of TN dissolved were determined by high performance liquid chromatography (HPLC).

RDC Method: In this method, the sample is placed with the external phase in the cell covered with a filter, and the amount of drug expelled from the rotating cell into the external phase is determined. The JP standard apparatus was used with an RDC attached. Flasks and other apparatus were conventional ones which met the JP standards. The dissolution rates at each point were measured by HPLC, similar to the PD method.

Cells: The filters used were a hydrophilic filter (HVLP) and a hydrophobic filter (HVHP) produced by Millipore.

As the internal phase, the 1st (pH 1.2) and 2nd (pH 6.8) fluids in the disintegration test in the JP and an acetic acid buffered solution, pH 4.0, were used at volumes of 2.5—10.0 ml. The cell rotation rates were 50, 100 and 150 rpm.

Test Solutions: In the external phase, *n*-octanol was used in addition to the pH 1.2, 4.0 and 6.8 solutions that were used in the internal phase.

Bioavailability Administration to Humans: Fourteen healthy male

TABLE I. Contents of Sample for Testing

Ingredients and quantities	
Contains:	
dl-α-TN	200.0 mg
Vehicle	
JP soybean oil	70.0 mg
JP polysorbate 80	10.0 mg
Fill weight	280.0 mg
Capsule film:	
JP gelatin	145.3 mg
JP concentrated glycerin	36.0 mg
JP D-sorbitol (70%)	7.1 mg
JP ethyl *p*-hydroxybenzoate	0.5 mg
JP propyl *p*-hydroxybenzoate	0.2 mg
JP titanium oxide	0.9 mg
Coloring agent	q.s.
Total weight	470.0 mg

volunteers (age 20—26 years, body weight 55—71 kg) were subjects of the study. They were prohibited from taking any other drugs from 1 week before initiation of the study until it was ended, and fasted from the day before the study. Thirty minutes after a meal, 1 capsule of TN-SC was given orally and the subjects then fasted for 4 h thereafter.

Methods of Blood Sampling and the Preparation of Blood Specimens: Before and 2, 4, 5, 6, 7, 9, 12 and 24 h after drug administration, 7 ml of blood was taken from the cubital vein with a heparin-containing vacuum blood sampler, mixed by shaking it upside-down, and centrifuged at 300 rpm for 10 min. The resultant plasma was immediately frozen and stored at −20 °C or lower temperature until analysis.

Assay of TN in Plasma: To 1.0 ml of plasma were added 0.2 ml of ethanol and 1.0 ml of methanol; this was mixed for 30 s in a vortex and 5.0 ml of hexane was added. After extraction under reciprocal shaking at 250 rpm for 5 min, the mixture was centrifuged at 1000 rpm for 5 min. Next, the hexane layer was concentrated and dried under a nitrogen gas flow (40 °C). This dried material was dissolved with 200 μl of isopropyl alcohol, and a 50 μl portion was subjected to HPLC. The TN content was calculated by the absolute calibration method. The conditions were: column, μ-bondapack C_{18}, i.d. 3.9 mm × 30 cm (Waters); guard column, Bondapack C_{18}/corasil (Waters); mobile phase, a mixture of acetonitrile–acetic acid–water (85:75:75); flow rate, 3.0 ml/min; column oven temperature, 35 °C; detection, UV 263 nm; and sensitivity, 0.01 a.u.f.s.

Results and Discussion

First, the solubility of TN for each test solution was examined. As Table II shows, TN was insoluble in the buffer solution but was freely soluble in the *n*-octanol solution. Therefore, the following experiments were performed.

PD Method First, in the PD method as described in the JP, we used two solutions, pH 1.2 and pH 6.8. No increase in dissolution rate was observed in either case. This was basically due to the insolubility of TN in the aqueous solution, which was the expected result but the test was performed to confirm this property. Actually, when TN-SC was put into the dissolution test flask, the SC soon disintegrated but the content accumulated on the surface of the test solution resulting in oily drops; this was attributed to the low solubility of TN in the test solution. In sampling the dissolved solution, we were careful not to collect any of these oily drops, but, as Fig. 1 shows, the dissolution rate resulted in a non-cumulative curve.

RDC Method The dissolution profile for each buffer solution was examined. A pH 1.2 buffer solution was used as the internal phase of the cell. As Fig. 1 shows, the dissolution was low as true with the PD method. This is also considered to be due to the low solubility of TN. On the basis of our past experience, we decided that a higher dissolution rate could not be obtained in the buffer solution.

Therefore, the following experiment was performed by the RDC method, in which the inside of the cell is regarded as the digestive tract and the outside of the cell as the tissue. A pH solution was used in the internal phase and *n*-octanol, which is often used for calculating oil–water distribution coefficients, was used in the external phase as a model organic solvent when a drug is absorbed inside the body. Of course, the drug absorption mechanism is complicated and cannot be explained by a single methodology. However, after the drug dissolves into a liquid state in the digestive tract, the solubility in fat expressed by the dissociation constant or the oil–water partition coefficient is a particularly important factor. Therefore, *n*-octanol was considered a more useful solvent than other organic solvents. Furthermore, it is notable that by the present *n*-octanol method a linear relationship was obtained between the *in vitro*/*in vivo* data in the tests on the slow-releasing SC[5] and water-based SC we devised.[1]

First, the influence of the pH in the internal phase was examined. As shown in Fig. 2, the dissolution rate was nearly 100% at both pHs, and thereafter, this pH 1.2 solution was used as the internal phase.

Next, the effects of different rotation rates, 50, 100

TABLE II. Solubility of TN for Each Test Solution after Shaking at 20 °C

Test solution	TN solubility (g/100 g mixed solution)
pH 1.2 buffer	<1
pH 4.0 buffer	<1
pH 6.8 buffer	<1
n-Octanol	>99

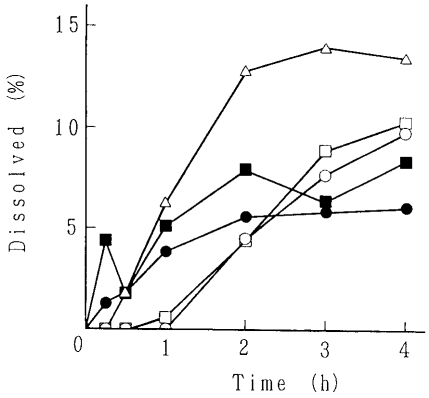

Fig. 1. Dissolution Profiles Obtained by PD Method (1000 ml, 100 rpm) and RDC Method (HVHP, 2.5 ml, 150 rpm) Using Test Solutions at Various pHs (HVHP, 2.5 ml, 150 rpm)

●, PD method, pH 1.2; ■, PD method, pH 6.8; ○, RDC method, pH 1.2; △, RDC method, pH 4.0; □, RDC method, pH 6.8.

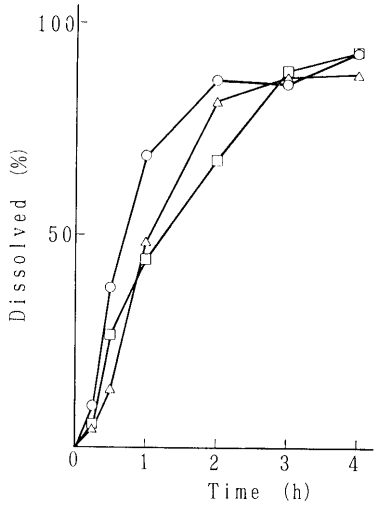

Fig. 2. Dissolution Profiles Obtained by RDC Method Using Internal Phases at Various pHs

HVHP, external phase was *n*-octanol, 2.5 ml, 50 rpm. ○, pH 1.2; △, pH 4.0; □, pH 6.8.

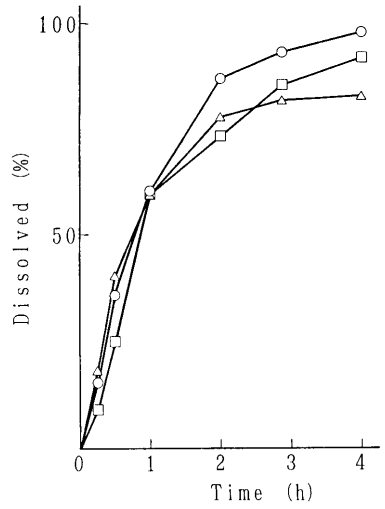

Fig. 3. Dissolution Profiles Obtained by RDC Method at Various Cell Rotation Rates

HVHP, external phase was *n*-octanol, pH 1.2, 2.5 ml. ○, 50 rpm; △, 100 rpm; □, 150 rpm.

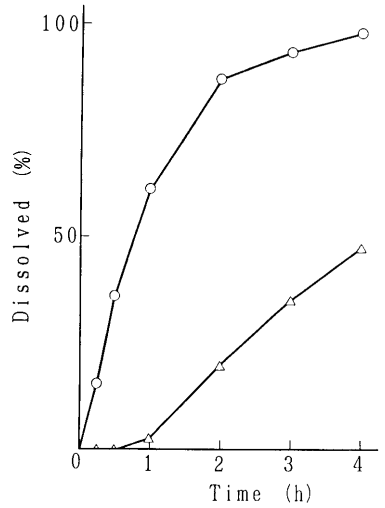

Fig. 4. Dissolution Profiles Obtained by RCD Method Using Different Filters

External phase was *n*-octanol, pH 1.2, 2.5 ml, 50 rpm. ○, HVHP; △, HVLP.

and 150 rpm, on the dissolution rate were examined. As shown in Fig. 3, similar dissolution profiles were found at all rotation rates. Since an oily solvent was used in the external phase, the use of HVHP appeared preferable, but to confirm this assumption, HVLP was also tested. As Fig. 4 shows, the dissolution rate with HVLP was only about 1/2 that obtained with HVHP. We considered that since the filter was hydrophilic, the pores were wetted and filled with the pH solution which acted as a barrier, inhibiting the transfer of TN to the oily *n*-octanol side (external phase). Next, the effect of the difference in the volume of the internal phase on the dissolution rate was examined. As Fig. 5 shows, the dissolution rate showed a trend of 2.5 ml, 5.0 ml > 10.0 ml. The smaller the volume of the internal phase, the larger the difference of the concentration gradient with the external phase, which accelerated the drug transfer.

Regarding bioavailability, the blood concentration of TN-SC after being given orally to humans was examined. Figure 6 and Table III shows the results.

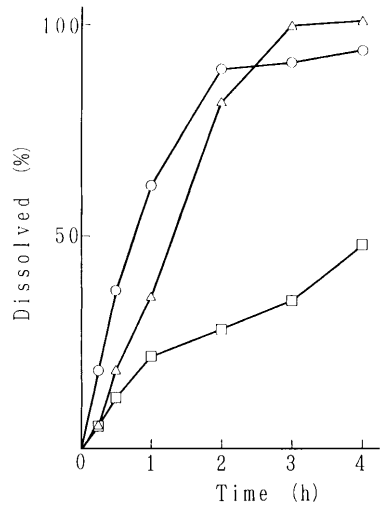

Fig. 5. Dissolution Profiles Obtained by RDC Method at Various Internal Phase Volumes

HVHP, external phase was *n*-octanol, pH 1.2, 50 rpm. ○, 2.5 ml; △, 5.0 ml; □, 10.0 ml.

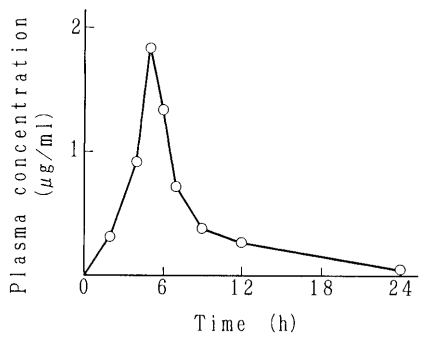

Fig. 6. Plasma Concentration of TN after Oral Administration in Humans

TABLE III. Pharmacokinetic Parameters of TN

AUC (0—24)[a] (μg/ml·h)	C_{max}[b] (μg/ml)	T_{max}[c] (h)	*MRT*[d] (h)	*VRT*[e] (h^2)	$T_{1/2}$[f] (h)
9.60±3.33	1.96±0.65	5.14±0.36	7.97±1.18	28.07±6.29	5.53±0.82

$n=14$, mean±S.D. *a*) Area under the plasma concentration–time curve for 0—24 h. *b*) Maximum plasma concentration. *c*) Time of maximum plasma concentration. *d*) Mean residence time. *e*) Variance of residence time. *f*) Biological half life.

Examination of the relationship between the *in vitro* and *in vivo* findings showed that the blood concentration had a slow rising curve (Fig. 7). To obtain the corresponding *in vitro* pattern, an *in vitro* test was performed based upon the results so far obtained. The test was made using the HVHP filter, 10 ml of pH 1.2 buffer in the internal phase, the cell rotation rate of 50 rpm, and 1000 ml of *n*-octanol in the external phase. As shown in Fig. 7, the findings showed a good correlation to the *in vivo* results.

In conclusion, valuable information was obtained by the dissolution test performed on TN-SC, which was regarded as a representative model of SC filled with an oily drug in an oily base. In the dissolution test, the SC capsule film is disintegrated in an aqueous test solution, but the test solution and SC content separate which makes the test difficult, since the SC contents are generally oily. To solve this problem, organic solvents have been used

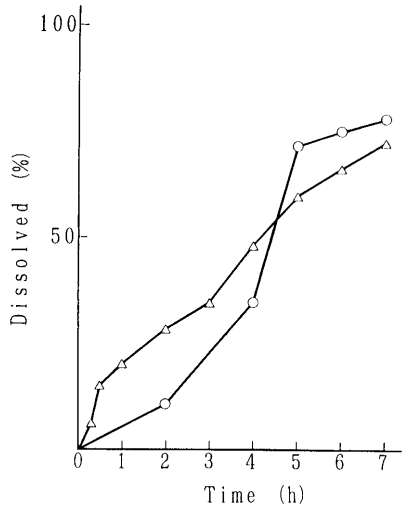

Fig. 7. Dissolution Profiles Obtained by RDC Method and Profiles with Deconvolution of *in Vivo* Data

HVHP, external phase was *n*-octanol, 10.0 ml, pH 1.2, 50 rpm. ○, *in vivo*; △, *in vitro*.

as the external phase, but then it is very difficult to disintegrate the capsule and to further dissolve the contents of the drug.

In the dissolution test performed by the PD method, it is difficult to depict various patterns because the number of factors is too small to adequately control the dissolution pattern. That is a problem when the correlation between dissolution *in vitro* and *in vivo* is examined on an SC whose time to reach its peak concentration is slow, such as the TN-SC. However, in the RDC method used in the present study, it is possible to separate the internal and external phase conditions. That is, the RDC method uses two main compartments, one is to disintegrate the capsule inside the cell, which mimics the function of the digestive tracts, and the other is to filter the drug out of the cell, which is another biological function of dispersing and absorbing the drug. Therefore, the method seems to be suitable not only for SC but also for dissolution tests of oily drugs. In the present study, when an aqueous solution was used as the test solution in the external phase, only low dissolution rates could be obtained by the RDC method and the PD method. Dissolution was improved, however, by the use of *n*-octanol. As the use of *n*-octanol in routine work may be difficult, it may be substituted by a test water solution containing a vegetable oil or a surfactant with a coefficient of distribution similar to *n*-octanol. From the standpoint of those who are routinely manufacturing SC, and in view of the continued increase of the various kinds, it is important to establish a fairly reasonable dissolution test for SC. At present, the RDC method is considered to be one of the best methods.

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References and Notes

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