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# Dissolution of Slightly Soluble Drugs. VI.[1] Effect of Particle Size of Sulfadimethoxine on the Oral Bioavailability[2]

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The effects of the particle size of sulfadimethoxine on its *in vitro* and *in vivo* dissolution rates and on bioavailability were examined using rabbits. The particle size significantly affected the rate and extent of bioavailability, and the *in vivo* dissolution, which was calculated by the deconvolution method. A good correlaion between *in vitro* and *in vivo* dissolution rates was obtained.

**Keywords**——sulfadimethoxine; bioavailability; particle size; *in vitro-in vivo* correlation; insoluble drug; deconvolution

A study of the general relationship between drug solubility and bioavailability revealed that particle size appreciably affected the rate of bioavailability when the solubility (in distilled water at 37°) ranged from 0.01 to 0.1%.[1] This finding was based on the assumptions that the absorption rate of highly lipid-soluble drugs in solution form remains maximal due to physiological factors such as the gastric emptying rate, the presence of an unstirred layer at the absorbing membrane surface, and capillary blood flow at the absorption site. In the present study we attempted to confirm our earlier findings[1] with additional results regarding the dissolution and absorption of sulfadimethoxine, which belongs to the practically insoluble or insoluble class.[4]

## Experimental

**Particle Preparation**——Commercial sulfadimethoxine was recrystallized from EtOAc, and sieved through a Ro-Tap testing sieve shaker (Japan Industrial Standard (JIS) sieves); 4 different particle sizes, 170—200, 80—100, 42—48, and 24—28 mesh, were used for the experiments. The mean diameter of the sieved particles is taken as the arithmetic mean diameter of the sieve opening except for 170—200 mesh. The *in vitro* dissolution test showed a high dissolution rate for the 170—200 mesh particles (Fig. 3, shown later), but microscopic observation showed a wider size distribution ascribable to the sieving after slight trituration of large particles. Therefore, to investigate what particle size corresponds to the 170—200 mesh sample in connection with the relation between particle sizes of samples obtained without trituration and their dissclution rate constants, a sample of 115—150 mesh (115 μm) was also tested. A logarithmic plot for particle sizes of 115, 163, 324, and 650 μm *vs.* the dissolution rate constants was constructed and the linear portion was extrapolated back to obtain the value of the dissolution rate constant of 170—200 mesh particles; the particle size corresponding to 170—200 mesh was determined to be about 33 μm. Thus, this value was adopted. A ground sample was obtained by ball-milling as described previously[5]; the mean surface diameter, determined by the air permeability method, was about 1.19 μm at a density of 1.56.

Each sample (200 mg) was prepared as described previously.[1] A suspension in 20 ml of water was orally administered to rabbits through a catheter. An aqueous solution (200 mg/20 ml) was prepared by dissolving Na-sulfadimethoxine in distilled water and administered orally as the standard reference.

1) Part V: N. Kaneniwa, N. Watari, and H. Iijima, *Chem. Pharm. Bull.*, **26**, 2603 (1978).
2) A part of this work was presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April 1978.
3) Location: *a) Hatanodai, Shinagawa-ku, Tokyo, 142 Japan*; *b) Hongo, Bunkyo-ku, Tokyo, 113 Japan.*
4) Definition of solubility of the Japanese Pharmacopeia.
5) N. Kaneniwa and N. Watari, *Chem. Pharm. Bull.*, **25**, 867 (1977).

**Animal Studies**——Four male albino rabbits, weighing around 2.5 kg, were used after fasting for 48 hr.[1] Each rabbit received the drug intravenously in the first week, and was given the aqueous solution of the drug in the second week. The 4 different particle sizes were administered to each rabbit at 7-day intervals, beginning with the smallest size. The aqueous solution and the largest sample size (650 μm) suspension were also tested with bile duct-ligated rabbits. After the experiment, the bile duct-ligated rabbits were autopsied to confirm that the ligation was complete.

Blood specimens (0.5 ml) were taken from the aural vein at intervals. Urine was collected up to 96 hr after the medication. Feces were also collected every 24 hr for 4 days.

**Identification and Determination of Sulfadimethoxine in Blood, Urine, and Feces**——Identification of unchanged sulfadimethoxine and its metabolites ($N^4$-acetylated and $N^1$-glucuronide) in the urine and feces was done by thin-layer chromatographic (TLC) analysis according to Uno *et al.*[6] $N^4$-Acetylsulfadimethoxine was the main metabolite; the amount of $N^4$-glucuronide recovered was very small.[7]

Sulfadimethoxine[8] in blood, urine, and feces was determined as "$N^4$-free" (direct reacting) and "total" ("$N^4$-free" plus $N^4$-acetylated) sulfadimethoxine by the Bratton-Marshall method. To analyze sulfadimethoxine in the feces, 10 volumes of distilled water were added to the dried feces and the pH of the solution was adjusted to approximately 10 with 2 N NaOH solution. About 10 min later, the mixture was homogenized and filtered. A part of the filtrate was centrifuged at 12000 rpm and the supernatant was appropriately diluted with distilled water, then assayed. The amount of the drug excreted in the feces was calculated from the amount of distilled water added.

***In Vitro* Dissolution Rate and Solubility**——The same apparatus and procedure as reported earlier[9] were used for the determinations of the amount of sulfadimethoxine dissolved in the medium and of the dissolution rate.

**Calculation of Bioavailability**——The time course of sulfadimethoxine in the blood after bolus intravenous administration could be described for all the rabbits by a three-term exponential equation, that is, $C=Pe^{-\pi t}+Ae^{-\alpha t}+Be^{-\beta t}$ $(\pi>\alpha>\beta)$. Here, $P$, $A$, and $B$ are constants and $\pi$, $\alpha$, and $\beta$ are the hybrid rate constants. The area under the blood concentration-time curve (AUC) after bolus intravenous administration was calculated as $P/\pi+A/\alpha+B/\beta$. The AUCs for procedures other than bolus intravenous administration were calculated using the trapezoidal method. The area for the tail end was evaluated as $Ct/\beta$ where $Ct$ is the blood concentration at the last time point $t$.

**Calculation of *in Vivo* Dissolution**——For *in vivo* dissolution calculation, the computer programs used were CMNLR2[10] for nonlinear regression analysis and a deconvolution program[11] in which the anterior phenomenon was approximated by an appropriate exponential polynomial and the deconvolution operation was done from the posterior phenomenon by the point-area method. The time ($T_{50}$) required for 50% of the drug to become dissolved was determined from the integrated weight function data for *in vivo* dissolution. The computation was carried out on a HITAC 8800/8700 digital computer at the University of Tokyo.

## Results and Discussion

### Time Course of Sulfadimethoxine in the Blood after Ingestion and Bioavailability

Fig. 1 shows the time course of sulfadimethoxine in the blood after oral administration of different particle sizes. The particle size appreciably affected blood levels, indicating that the dissolution process was rate-limiting in the absorption.

Table I compares the pharmacokinetic parameters for bioavailability among different particle sizes; statistically significant differences exist between the aqueous solution and particle sizes of 324 and 650 μm as regards AUC. There was a qualitative linear relation between particle size and the blood level peak time ($T_{max}$). The peak blood level ($C_{max}$) significantly decreased with increasing particle size.

As is clear from the 5 th column of Table I, there were statistically significant differences in the amount excreted in the feces between the aqueous solution and particle sizes of 324 and 650 μm. The amount of the drug excreted increased with increasing particle size. Excretion of sulfadimethoxine in the feces was found mainly during the first 24 hr and hardly at

6) T. Uno, T. Kushima, and T. Hiraoka, *Chem. Pharm. Bull.*, **15**, 1272 (1967).
7) J.W. Bridges, M.R. Kibby, and R.T. Williams, *Biochem. J.*, **91**, 12p (1964).
8) Dissociation constants; $pKa_1$ 2.02 and $pKa_2$ 6.05.
9) N. Kaneniwa and N. Watari, *Chem. Pharm. Bull.*, **22**, 1699 (1974).
10) H. Kiwada, K. Morita, M. Hayashi, S. Awazu, and M. Hanano, *Chem. Pharm. Bull.*, **25**, 1312 (1977).
11) OS7 Hitachi Statistical Analysis Program, 8700-7-004-02, Tokyo, Japan 1973.

all during the following 24 hr. The amount of sulfadimethoxine determined as $N^4$-free drug was consistent with that of total drug within the range of experimental error. TLC analysis confirmed that only unchanged sulfadimethoxine was present in the feces.

To investigate the unchanged sulfadimethoxine excretion in the feces, the aqueous solution and the largest sample size of 650 μm were also tested under the same conditions with bile duct-ligated rabbits, and the results are shown in Table II. The amount of unchanged sulfadimethoxine excreted in the feces was similar to that in normal rabbits, confirming that the excretion of unchanged drug in the feces was not due to biliary excretion but to passage of the drug through the gut because of its very poor aqueous solubility.

*In vivo* drug dissolution was calculated from the deconvolution between blood levels after the administration of aqueous solution and the administration of each powder sample. The blood level after oral administration of aqueous soluton was standardized as the anterior phenomenon. Fig. 2 shows the time course of the integrated weight function for *in vivo*

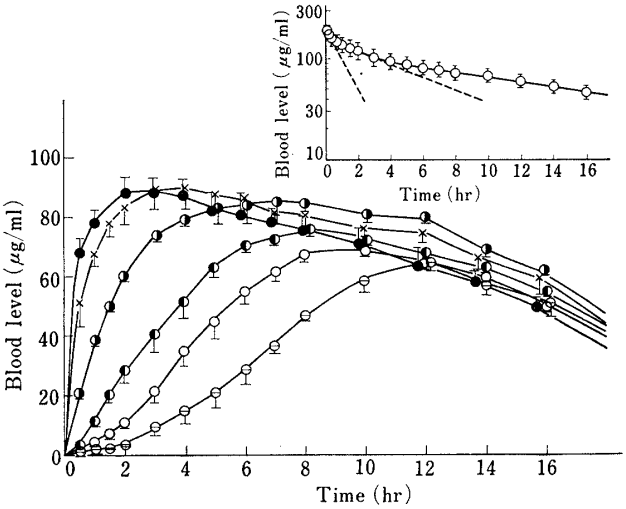


Fig. 1. Effect of Particle Size of Sulfadimethoxine on Blood Level

Data are the averages of four rabbits with the standard error, ● soln., × ball-milled, particle size (μm) ◑ 33, ◐ 163, ○ 324, ⊖ 650.
The inset graph shows the time course of sulfadimethoxine in the blood after bolus intravenous injection.

TABLE I. Pharmacokinetic Parameters for Bioavailability

| Sample | AUC[a] (μg·hr/ml) ×10⁻² | $T_{max}$[b] (hr) | $C_{max}$[a] (μg/ml) | In feces (%)[a] | | In urine during 96 hr (%)[a] | |
|---|---|---|---|---|---|---|---|
| | | | | $N^4$-Free | Total | $N^4$-Free | Total |
| *i. v.* injection | 21.0±1.9 | — | — | — | — | 70.2±2.3 | 93.2±5.0 |
| Soln. | 20.5±1.3 | 3.0(2.0—4.0) | 91.0±4.4 | 1.9±0.8 | 2.0±0.5 | 71.4±6.8 | 89.6±6.5 |
| Ball-milled | 22.8±1.5 | 4.0(3.0—5.0) | 90.9±6.9 | 2.6±0.7 | 3.4±0.8 | 54.6±4.9 | 89.1±3.4 |
| 33 μm | 22.7±0.5 | 6.8(6.0—7.0) | 86.5±3.8 | 4.3±0.5 | 5.8±0.5 | 53.7±2.4 | 70.3±2.9 |
| 163 μm | 19.0±0.4 | 7.8(7.0—8.0) | 75.2±0.8* | 7.6±1.1* | 8.6±1.2 | 38.4±4.2 | 60.4±6.4 |
| 324 μm | 16.6±0.5* | 9.5(9.0—10) | 69.5±1.4** | 12.8±1.2** | 13.2±1.3 | 28.4±2.4 | 46.0±3.1 |
| 650 μm | 15.2±1.0** | 11.5(10—12) | 65.2±3.3** | 23.4±1.2** | 24.3±1.2 | 17.7±1.9 | 30.6±4.6 |

*a*) Mean±standard error. *b*) The range of peak time is given in parentheses.
The significances of differences between aqueous solution and each sample, using Student's *t* test, are as follows: *$p<0.05$ and **$p<0.01$.

TABLE II. Amount of Sulfadimethoxine excreted in the Bile Duct-ligated Rabbits

| Sample | In feces (%) | | In urine during 96 hr (%) | |
|---|---|---|---|---|
| | $N^4$-Free | Total | $N^4$-free | Total |
| Soln. | 0.6±0.4 | 0.7±0.1 | 23.9±5.7 | 71.1±9.3 |
| 650 μm | 25.3±5.0 | 25.0±4.6 | 17.9±4.6 | 36.2±2.9 |

Each value is the mean of three rabbits with the standard error.

dissolution. It is clear that particle size appreciably affected the total amount dissolved. There were statistically significant differences ($p<0.01$, Student's $t$-test) between the ball-milled sample and particle sizes of 324 and 650 μm as regards the total amount of the drug dissolved. When the ratio of the total amount dissolved to the dose administered and the excretion ratio in the feces were added, the sum was reasonable in relation to the unitary amount administered for each sample. As shown in Fig. 2, $T_{50}$ increased with increasing particle size, and the time required to reach the plateau level for *in vivo* dissolution approximately corresponded to the $T_{max}$ for each sample.

A good correlation was found between the AUC and the maximum integrated weight fucntion value, but not between each of them and the amount excreted in the urine 96 hr after medication. This may be because sulfadimethoxine and its metabolites were not excreted completely within 96 hr, as reported earlier.[6] Furthermore, the amount not excreted in the urine may increase with decreasing absorption rate.

## Relationship between *in Vitro* and *in Vivo* Dissolution Rates

*In vitro* dissolution was tested under sink conditions in distilled water and 0.1 N HCl solution. The test was performed at 700 rpm so as to obtain thoroughly wetted and completely dispersed powder particles.[9] Fig. 3 shows a plot based on the Hixson-Crowell cube root law in distilled water. The ratio of dissolution rate constant in 0.1 N HCl solution to

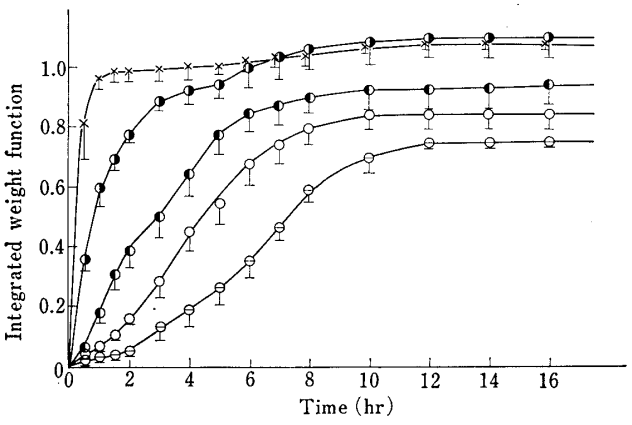


Fig. 2. Time Course of the Integrated Weight Function for *in Vivo* Dissolution

The symbols are the same as in Fig. 1.

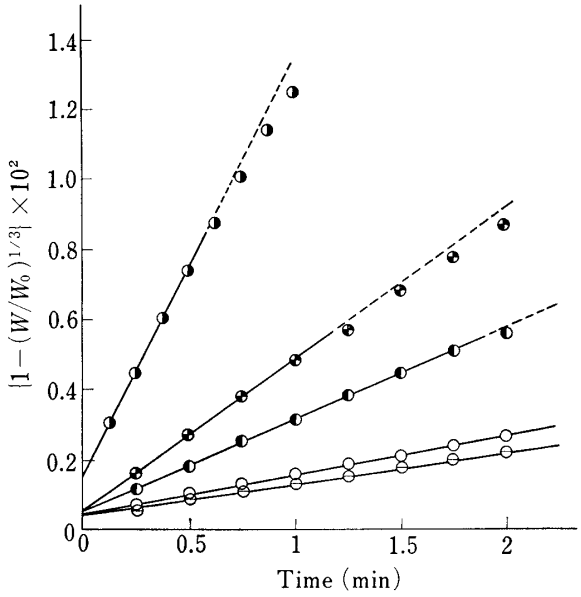


Fig. 3. Plot based on the Hixson–Crowell Cube Root Law in Distilled Water

◑ 170—200 mesh, ◒ 115—150 mesh, ◐ 80—100 mesh, ○ 42—48 mesh, ⊖ 24—28 mesh.

Table III. Comparison of *in Vitro* Dissolution Rate Constants and Solubilities in Two Different Media

| Medium | Dissolution rate constant ($min^{-1}$) $\times 10^3$ | | | | | Solubility [a] (mg/100 ml) |
|---|---|---|---|---|---|---|
| | 33 μm | 115 μm | 163 μm | 324 μm | 650 μm | |
| Distilled water | 12.2 | 4.32 | 2.54 | 1.11 | 0.843 | 4.63 |
| 0.1 N HCl solution | 49.7 | 16.6 | 10.1 | 4.57 | 3.15 | 24.6 |
| Ratio[b] | 4.07 | 3.85 | 3.97 | 4.12 | 3.74 | 5.31 |

a) Measured at 37°. b) The value in 0.1 N HCl solution divided by that in distilled water.

that in distilled water was almost constant for each sample, but a larger solubility ratio was noted, as shown in Table III. This difference of ratio between dissolution rate and solubility may explained by reasoning similar to that of Gibaldi.[12]

To facilitate correlations between *in vitro* and *in vivo* dissolution rates, the *in vivo* parameters for $T_{50}$ (mean value of four rabbits) or $T_{max}$ were plotted against *in vitro* $T_{1/2}$ determined in 0.1 N HCl solution (Fig. 4). There were good linear relationships between *in vitro* and *in vivo* parameters, and the correlation coefficients are 0.990 between $T_{50}$ and $T_{1/2}$, and 0.995 between $T_{max}$ and $T_{1/2}$. Furthermore, the slopes of these two lines are very similar and more than unity, indicating that the *in vivo* dissolution was very slow. This differences between *in vitro* and *in vivo* dissolution rates may be primarily ascribable to the agitation, because the high agitation speed of 700 rpm was used in the *in vitro* dissolution test.

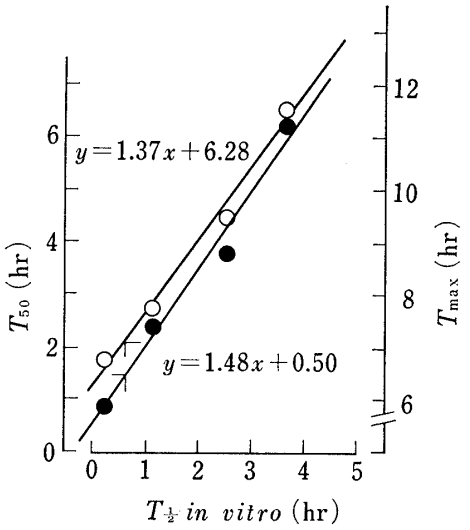


Fig. 4. Relationship between *in Vitro* and *in Vivo* Dissolution Rates

●, $T_{50}$ for *in vivo* dissolution.
○, $T_{max}$ for peak blood level.

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12) M. Gibaldi, "Biopharmaceutics and Clinical Pharmacokinetics," 2nd ed., Lea and Febiger, Philadelphia, 1977, p. 32.