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# Bioavailability of different artemisinin tablet formulations in rabbit plasma—correlation with results obtained by an in vitro dissolution method

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**Abstract**

The demonstration of a good overall correlation with in vivo data is the ultimate proof of qualification for any dissolution-rate test. For artemisinin, a very hydrophobic compound at a high content in oral solid dosage forms, all official dissolution apparatus were estimated unsuitable. A modified two phase partition-dissolution method was applied to solve this problem. This study reports on the bioavalability of three different formulations of artemisinin tablets in rabbit plasma. Artemisinin concentrations in plasma were determined by liquid chromatography. A linear correlation between results obtained by the partition-dissolution method described and the obtained in vivo data confirmed the validity of the dissolution method. © 1997 Elsevier Science B.V.

*Keywords:* Artemisinin; Bioavailability

## 1. Introduction

One of the most important as well as most difficult approaches in the biopharmaceutic evaluation of a drug is the quantitative correlation of absorption and in vitro kinetics. For immediate-release (IR) products, the in vitro dissolution test is considered as the single most important and useful quality control procedure; it can serve as an indicator of bioavailability only when appropriate in vivo/in vitro correlations exist [1].

Artemisinin, isolated from the herb *Artemisia annua* by Chinese scientists, is a leading compound of a new class of antimalarials [2,3]. Although several thousands of patients were saved by using artemisinin, and the commercial products are available worldwide [4], correlation between bioavalability in vivo and in vitro dissolution kinetics has not been studied yet.

In a previous report, it was shown that all official dissolution methods described in the USP [5] did not fulfil the requirement of sink conditions for artemisinin solid oral dosage forms [6]

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and therefore a two phase partition-dissolution method was applied [7]. To validate the in vitro method, in vivo experiments are required.

In this study, the bioavailability of three different formulations of artemisinin tablets was studied in rabbits, in order to verify the correlation between in vivo data and data obtained by the in vitro dissolution method.

## 2. Experimental

### 2.1. Materials

Artemisinin (ART) with a purity of 99.87%, was purchased from Mediplantex, Hanoi, Vietnam; tablet M2 (250 mg ART) is one of the commercial products available in Vietnam [6]; tablets FD4 and FD6 (200 mg ART) have been prepared as described previously [8].

Gelatine capsules (type 0) were obtained from UCB (Brussels, Belgium). Anticoagulant Acid Citrate Dextrose solution (A) (ACD Solution A) was purchased from Becton Dickinson, Rutherford, NJ, USA. Methanol and ethyl acetate were LC grade (Rathburn, Walkerburn, Scotland) and all other chemicals were reagent grade (Acros Chimica, Geel, Belgium). Deionised, distilled water was used in all the experiments.

### 2.2. Study design

To study the effects caused by formulation differences, three different formulations of ART tablets are used. As a measure of dissolution rate, the time for dissolution of 50% of the ART content ($T_{50}$), obtained by the two phase partition-dissolution method [7] is chosen. This value is plotted against the in vivo AUC value (Area Under the Curve, concentration versus time). A linear correlation between regression of AUC and $T_{50}$ is expected to prove the validity of the in vitro dissolution method applied.

Ten male, healthy Hollander rabbits (Animal Centre of Katholieke Universiteit Leuven, Belgium), with a weight of $1.93 \pm 0.12$ kg orally received ART at a dose of 400 mg. Tablets were broken into big granule form, amounts corresponding to 400 mg of ART were correctly weighed and put into gelatine capsules. This step was required because of the different ART content in different tablets. However, it did not influence the comparison between tablets because all tablets were treated in the same way and showed disintegration times of about 2 min. Between each administration a wash out period of 2 weeks was maintained. Blood samples were obtained by venipuncture in the ears and collected in tubes containing ACD Solution A. Blood samples were taken before treatment and at 10, 30, 60, 90 and 10 000 min (1 week).

### 2.3. Artemisinin assay

ART concentration was measured according to a slight modification of the method described by Zhao and Zeng [9]. After treatment of ART with NaOH, the resulting degradation product shows a UV maximum at 258 nm and can be determined by liquid chromatography (LC).

Plasma was obtained by centrifugation of the blood samples at 3500 rpm for 15 min and at 4°C. A volume of 700 μl plasma was pipetted into a clean reagent tube. After addition of 2.5 ml of ethyl acetate and vortexing during 1 min, the liquid phases were separated by centrifugation at 3500 rpm for 15 min. A volume of 2.1 ml of the organic phase was transferred into another tube and evaporated in vacuum at 60°C. The residue was dissolved in 100 μl of methanol. After addition of 200 μl of a 0.4% NaOH solution and mixing, the tubes were placed in a water bath at 30°C for 30 min. Then the samples were neutralised by adding 40 μl of 1 M $CH_3COOH$ in methanol and an amount of 200 μl was subjected to analysis by LC.

The LC system consisted of a Marathon autosampler (Spark Holland, Emmen, The Netherlands), a Merck-Hitachi L6200 pump (Darmstadt, Germany), a Waters Model 440 UV detector (Milford, MA, USA) set at 254 nm and a Hewlett Packard HP 3396 Series II integrator (Avondale,PA, USA). A laboratory-packed LC column ($250 \times 3.2$ mm) containing Hypersil C18 5 μm (Shandon, Runcorn, England) was equilibrated and eluted with a mixture of 0.01 M potassium

dihydrogen phosphate, adjusted to pH 4.5 with 0.1 M orthophosphoric acid, and methanol (58.5: 41.5, v/v) at a flow rate of 0.5 ml $min^{-1}$. Solutions obtained by extraction of plasma samples and reference solutions were analysed alternately.

## 3. Results and discussion

### 3.1. Optimization of the derivatization reaction

The concentrations of NaOH and $CH_3COOH$ used in the derivatization reaction are critical factors, since they influence both the completeness of the reaction and the stability of the resulting compound. In previous articles [10–12], different concentrations of NaOH (0.2 or 0.4%) and $CH_3COOH$ (0.1, 1 or 2.5 M) were used. Table 1 reports the final pH obtained with different concentrations of NaOH and $CH_3COOH$ in a mixture containing 200 μl of NaOH, 100 μl of MeOH and 40 μl of $CH_3COOH$. Since the UV absorbing derivatization product with absorption maximum at 258 nm is stable at neutral pH, mixtures of 1 M or 2.5 M $CH_3COOH$ with 0.2 or 0.4% NaOH seem to be suitable. When these conditions were used for the derivatization of ART, no significant differences in the peak height were observed (data not shown). The 0.4% NaOH and 1 M $CH_3COOH$ were finally retained.

Temperature and reaction time with NaOH are also important factors. In the original assay method described by Zhao and Zeng [9], the reaction occurred at 50°C for 30 min. Titulaer et al. [11] and Augustijns et al. [12] used slightly modified conditions: 45°C during 30 min and

Table 1
pH obtained during derivatization with different concentrations of NaOH and $CH_3COOH$

| | 0.2% NaOH | 0.4% NaOH | 0.8% NaOH |
|---|---|---|---|
| 0.1 M $CH_3COOH$ | 12.34 | 12.72 | 13.07 |
| 1 M $CH_3COOH$ | 5.28 | 6.41 | 10.7 |
| 2.5 M $CH_3COOH$ | 5.12 | 5.71 | 8.61 |

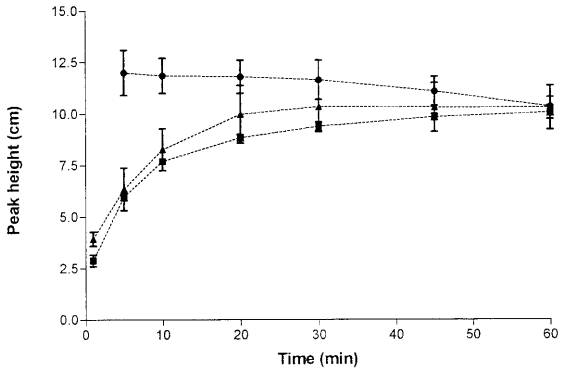


Fig. 1. Influence of reaction time and temperature of the derivatization reaction with NaOH on the peak height in the chromatogram ■: room temperature; ▲: 30°C; ●: 50°C

50°C during 5 min. In this study, the reaction was carried out at room temperature, 30 and 50°C and for different reaction times. The results are shown in Fig. 1. The mean value ($\pm 1$ S.D.) of three experiments is reported. At 50°C, the peak height continuously decreased in function of time, due to decomposition. At room temperature, it increased without reaching a constant level. At 30°C, a plateau was reached after 30 min. Therefore, 30°C and 30 min were retained as the final conditions.

### 3.2. Assay performance

The repeatability expressed as the relative standard deviation (RSD) was 2.1% ($n = 6$). The detection limit was about 5 ng $ml^{-1}$ (expressed as the signal-to-noise ratio (S/N) of 3.7). In order to construct a calibration curve, spiked plasma samples with final ART concentrations ranging from 20 to 400 ng $ml^{-1}$ were prepared. Therefore, a stock solution was made of about 50 mg ART, exactly weighed, in 50 ml of ethanol. This solution was further diluted so that small volumes (20–50 μl) had to be added to 700 μl of blank plasma. These spiked samples were extracted and analysed as described for the ART assay (see methods). As a reference to determine the extraction efficiency, solutions in alcohol, corresponding to the same final concentrations were analysed three times without carrying out the mixing with blank plasma and the extraction procedure. The mean results ($\pm 1$ S.D.) of three independent experi-

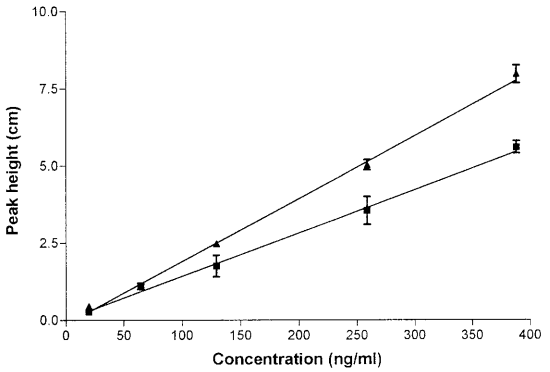


Fig. 2. Calibration curves obtained with and without extraction, showing the efficiency of recovery ■: after mixing with blank plasma and extraction; ▲: without mixing with blank plasma and extraction (corresponding to 100%)

ments are shown in Fig. 2. Both calibration curves show good linearity with correlation coefficients $>0.97$. A recovery of 79% was calculated from these results.

Fig. 3 shows a typical chromatogram of a rabbit plasma sample containing 130 ng $ml^{-1}$ ART.

### 3.3. Analysis of biological samples

#### 3.3.1. Bioavailability of different artemisinin tablet formulations in rabbit plasma

The plasma concentration-time curves obtained after administration of the different tablet formulations are shown in Fig. 4. Three rabbits were used for each formulation and each sample was analysed once. The mean value ($\pm 1$ S.D.) is reported. Differences are observed during the first 60 min. The concentration of artemisinin in plasma, after reaching the maximum value, rapidly dropped to a level around 50 ng $ml^{-1}$, and then slowly decreased to about 20 ng $ml^{-1}$ over 1 week. After 2 weeks, no artemisinin could be detected in the plasma any more with our system. The results allow to calculate AUC values by trapezoidal integration from 0 time to 90 min after drug administration.

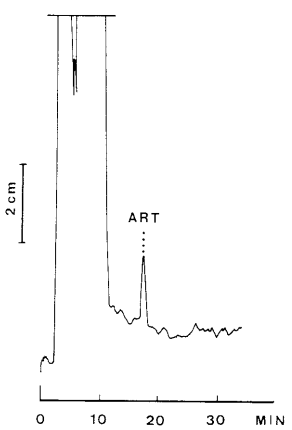


Fig. 3. Typical chromatogram of a rabbit plasma sample spiked with ART (130 ng $ml^{-1}$)

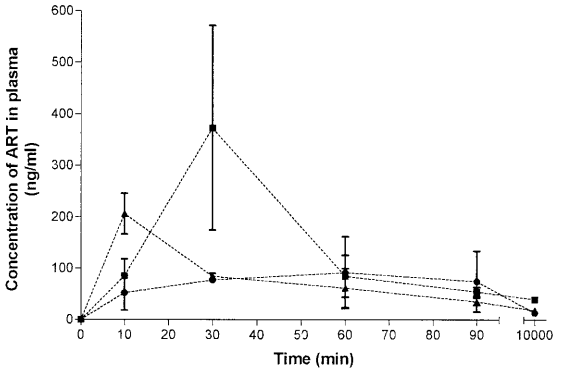


Fig. 4. Concentration of artemisinin in rabbit plasma after oral administration of different tablet formulations (400 mg ART) ■: FD4; ▲: FD6; ●: M2

#### 3.3.2. Relationship with the in vitro data obtained by using a modified partition-dissolution method

The in vitro dissolution results are illustrated in Fig. 5 as the rate of ART dissolution, obtained by using the two phase partition-dissolution method [7], plotted according to first-order kinetics. $T_{50}$ values of 1.56, 3.24, 3.62 h were calculated for the three formulations FD4, FD6, and M2, respectively [8]. If a linear relationship exists between $T_{50}$ values and AUC values, the in vitro method is said to exhibit correlation with the in vivo results [13]. AUC values were determined for each rabbit separately. Mean values ($\pm 1$ S.D.) are reported in Fig. 6, which shows a linear correlation between the mean AUC values and $T_{50}$ with a correlation coefficient of 0.997.

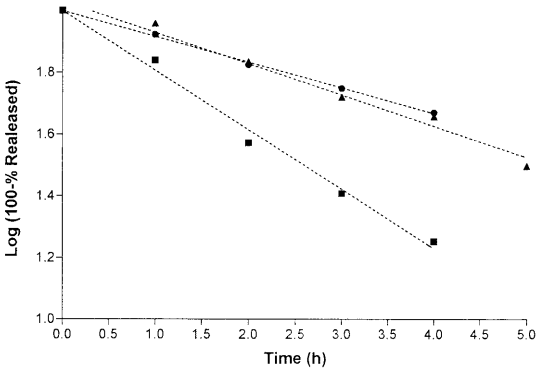


Fig. 5. Dissolution profile of different formulations of artemisinin tablets ■: FD4; ▲: FD6; ●: M2

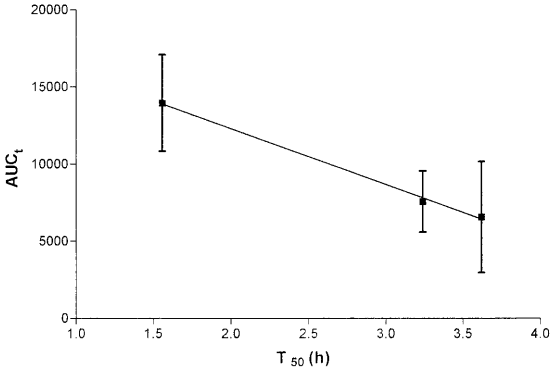


Fig. 6. Correlation between AUC (in vivo) and $T_{50}$ (in vitro) values for different tablet formulations

## 4. Conclusion

The good correlation between the bioavailability results in rabbits and the dissolution data obtained for the tablets does support the validity of the two phase partition-dissolution method used.

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