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# BIOAVAILABILITY OF FOUR OESTRADIOL SUSPENSIONS WITH DIFFERENT PARTICLE-SIZES - IN VIVO/IN VITRO CORRELATION

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## ABSTRACT

A comparative bioavailability study was performed, using three specially made particle-size fractions of oestradiol and a sample of oestradiol commercially obtained, all chemically equivalent. Aqueous suspensions of these four fractions were administered orally to five subjects, following a completely randomized cross-over design. The in vitro tests included particle-size analysis, using resistance particle counter and dissolution characterisation, using the Paddle method and also again the resistance particle counter method. The influence of particle size on the initial absorption is clearly significant ($p < .01$). The influence of particle size on total area (AUC) under the curve, show a weak significance ($p < .05$).
Both in vitro dissolution methods showed clearly different profiles for the four suspensions, which are in agreement with the differences, shown by the size frequency distribution.
A good correlation coefficient (0.983) are obtained

by comparing particle-size distribution with in vitro release data. In vivo/in vitro correlation was demonstrated by comparing values for AUC and % dissolved from in vitro data. The correlation coefficients was > 0.950.
The results clearly indicate the great importance of the particle-size distribution for dissolution profiles, and hence for the absorption.



## INTRODUCTION

Dissolution rate studies and bioavailability of drugs are important research areas. The in vitro dissolution tests are of great importance in predicting the in vivo course of a drug, when the correlation is known, as described previously, Strum et al. (1) and Shah et al. (2).
Particle-size reduction is a mean of increasing absorption rate and physiological availability, provided the dissolution rate of the drug is the rate-limited step, and provided the advantages obtained by using micronized drugs are not lost in the final dosage forms by inadequate formulation, as described by Fincher (3) and Levy (4).
Previous studies, Howard et al. (5) and Bates et al. (6), showed the necessity of determining the dissolution characteristics for suspensions.

An in vivo/in vitro investigation with oestradiol in different tablet formulations has recently been described by Johansen et al. (7).
In this report, the dissolution and particle-size profiles were determined in order to obtain information on the variation in particle characteristics directly

related to the dissolution profile. The reproducibility of the dissolution method was demonstrated by determining the dissolution profile of four different particle-size oestradiol suspensions.

This paper also reports on correlation studies with in vivo and in vitro data from both Paddle method and resistance particle counter method as well as particle-size measurements.

## MATERIALS AND METHODS

### Preparation of particle fractions and suspensions

The three special fractions were prepared from a commercial-quality un-milled oestradiol.
Fraction $F_I$ (smallest mean diameter) was prepared by wet-milling in a special ball-mill (8). $F_I$ was available only as suspension, since it was not possible to keep the particle size intact during the drying process.
Fractions $F_{II}$ (intermediate mean diameter) and $F_{III}$ (largest mean diameter) were prepared by mild grinding in a mortar, followed by a combination of wet sieving, gravitational and centrifugal sedimentation until the desired particle-size distributions were obtained, as shown in Figure 1. The two fractions were collected on a filter and dried.

micronized quality oestradiol.
Earlier studies reported that grinding processes may affect the crystallinity of oestradiol, Florence & Salole (9) and von Kuhnert-Brandstätter (10), therefore all the fractions were examined for changes in their crystallinity by X-ray diffractometry and differential scanning colorimetry. No changes or differences were found in any of the four fractions.

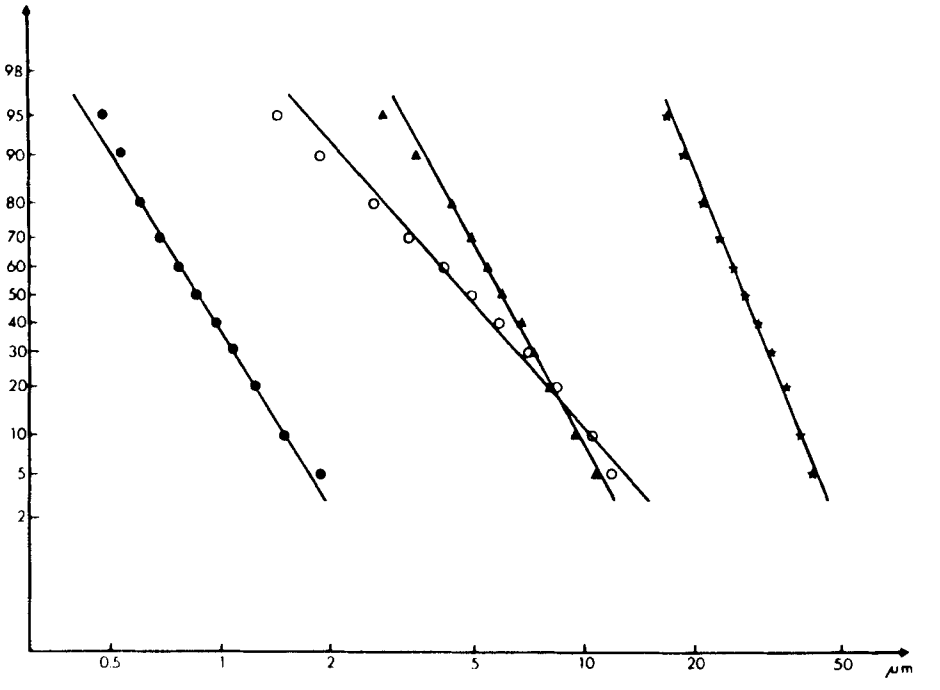


FIGURE 1:

Particle-size distribution of the four oestradiol fractions shown on log probability graph.

KEY: ● $F_I$ fraction, ▲ $F_{II}$ fraction, ★ $F_{III}$ fraction, ○ Standard fraction.

The four suspensions used in the in vivo/in vitro study contained 2 mg oestradiol per 10 ml suspension. The vehicle consisted of 0.1% polyvinylpyrrolidone as wetting agent and 0.1% sodium benzoate as preservative.

Particle-size analysis

All size distributions were analyzed in a resistance particle counter (11) with 30 µm, 50 um and a

140 μm aperture tubes. The calibration material for the different aperture tubes was 1.15 μm - 8.06 μm and 18.1 μm Latex beads, respectively. All the size distributions were measured with a reading of approx. 0.05 on the concentration meter to eliminate coincidence problems.

The electrolyte used for particle-size measurements was made of 1% sodium chloride saturated with oestradiol. The electrolyte was filtered to keep a low background count, usually approx. one hundred particles of 1.0 - 2.0 μm/ml for a 50 μm aperture tube.

In vitro dissolution - Paddle method - chemical analysis

The dissolution apparatus consisted of a 2000 ml three-necked round bottom flask (12) immersed in a water bath at 37°C ± 1°C. The flask was equipped with a stirrer with a half moon shaped teflon paddle of 19.7 $cm^2$ (radius 7.6 cm and chord 11.3) placed 2.5 cm above the bottom. The stirrer was driven by a standard servodyne power drive system (13) at 60 rpm. At 0 min, 20.00 ml of the suspensions containing 4 mg oestradiol were added with a full pipette through the left neck of the flask, 2 cm below the surface to 1980 ml of 0.1 N HCl at 37°C. At 15, 30 and 60 min. 100.00 ml samples were withdrawn and immediately replaced by an equal volume of the dissolution medium. The samples were filtered through a Millipore filter (14). 50.00 ml aliquots were evaporated and analyzed spectrophotometrically at 500 nm for oestradiol, according to the method described by Urbanyi & Rehm (15).

In vitro dissolution - Paddle method - resistance particle counter method

The apparatus was similar to the one described in the Paddle method above. Sampling time for fraction $F_I$ was changed to 5, 10, 15 and 30 min. because of the

very rapid dissolution rate for this fraction. The samples were withdrawn and analyzed immediately on the resistance counter. To obtain a zero value, with no oestradiol in solution, the first sample was withdrawn 60 sec. before adding the suspension. 60 sec. of stirring at 60 rpm is adequate for a homogenous distribution of the suspension in the medium.
The zero-sample was set manually to 100% cumulated volume distribution and all the following samples were measured in relation to the zero-sample.

In vivo studies - protocol

Five healthy postmenopausal women were treated in a double-blind cross-over study with 10 ml of suspensions containing 2 mg of oestradiol in the four different particle sizes, $F_I$, $F_{II}$, $F_{III}$ and Standard. The administration was followed by ingestion of 200 ml of water. All subjects fasted from the night before until four hours after administration. Blood samples were drawn at 0, 7½, 15, 30, 45, 60, 90 min. and 2, 4, 8, 12, 24, 48 and 72 hours, and the plasma was analyzed for oestradiol and oestrone.
The in vivo study has been described in details by Englund & Johansson (16).

## RESULTS

### Particle size of fractions and suspensions

Results of all particle-size measurements of the oestradiol fractions are shown in Figure 1.
It will be seen from Figure 1. that there was only a minimal overlap between the three fractions $F_I$, $F_{II}$ and $F_{III}$. These are narrow distributions in contrast to the Standard fraction, which shows wider distribution

From the size distribution data it is possible to calculate the surface area. The surface area is derived from the following calculation in case of log normal distribution - straight line cumulative weight per cent versus diameter plot on log probability axes - the so-called Hatch-Choate equation as described by Herdan (17)

$$\log d_s = \log d_w - 1.151 \log^2 \sigma_g$$

where $d_w$ is the weight mean diameter (the 50% size), $d_s$ the volume surface diameter, and $\sigma_g$ the geometric standard deviation.

The Hatch-Choate equation is used under the approximation, that we assume that all the particles have the same shape, which were assumed to be spherical for comparison purposes.

The specific surface area $S = \frac{6}{d_s \times \rho}$ $m^2/g$ where $\rho$ is the density of the powder. The density ($\rho = 1.23$ g/cm$^3$ is measured by means of an Beckman Air Comparison Pycnometer.

After preparation the suspensions were analyzed on the resistance particle counter in order to check on possible changes in the size distribution.

The results show a slight displacement of the size distribution towards the smaller size, as compared with the results from the fractionated oestradiols obtained before formulation. To examine if there were any change in the size distribution during the investigation period, a five month storage test was done. All the suspensions were stored at 4°C. No significant changes were seen during the storage period.

In vitro dissolution studies - Paddle Method

The dissolution profiles of the four suspensions are shown in Figure 2. The average per cent dissolved

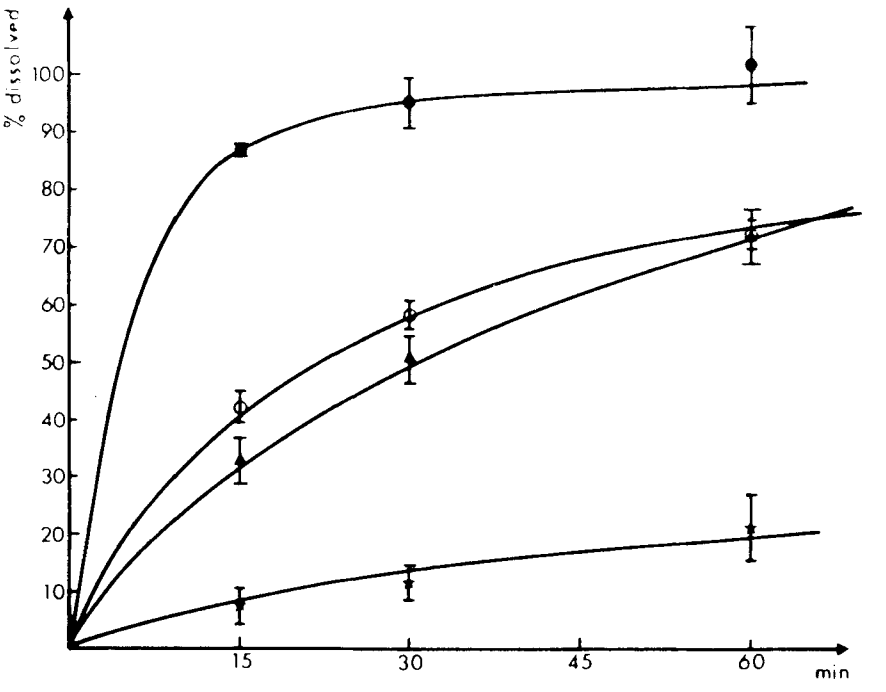


FIGURE 2:

The mean per cent dissolved (±s.d.) of the four oestradiol suspensions.

KEY: ● $F_I$ suspension, ▲$F_{II}$ suspension, ★$F_{III}$ suspension and ○Standard suspension.

oestradiol represents the mean values of 6 single determinations.

Suspension $F_I$ dissolved rapidly, compared to suspension $F_{III}$, which showed a slow dissolution rate. Suspension $F_{II}$ and Standard show equal dissolution profiles except for the first samplings, but both suspensions clearly differ from suspension $F_I$ and $F_{III}$.

In vitro dissolution studies - resistance counter method

The dissolution profiles for the four suspensions are shown in Figure 3. The average per cent undissolved oestradiol represents the mean of 6 single determinations. Suspension $F_{III}$ (coarse fraction) shows a very slow release, whereas suspension $F_I$ (fine fraction) shows a very rapid release in contrast to both $F_{II}$ (medium

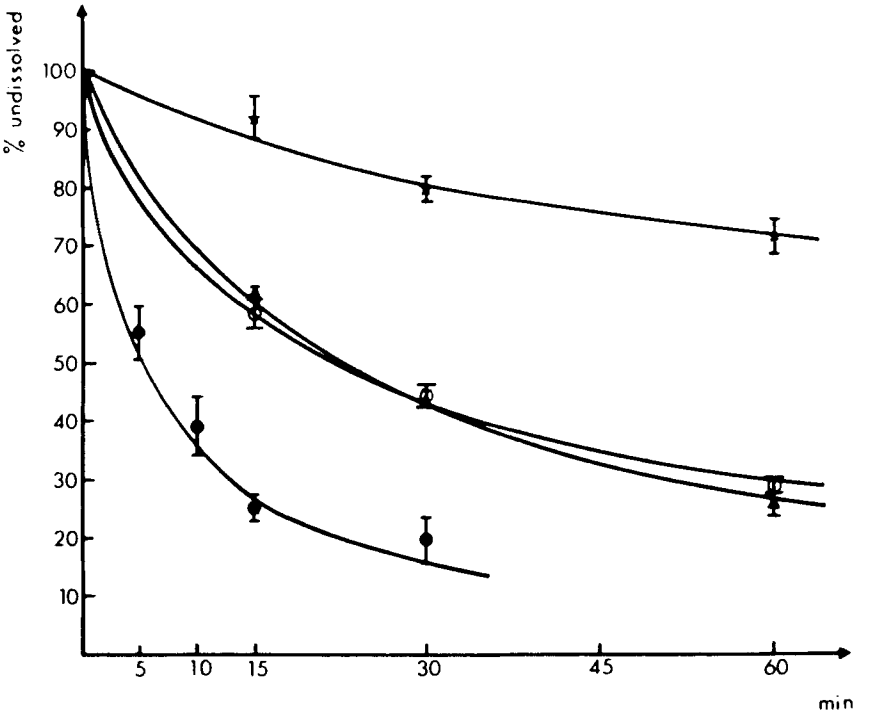


FIGURE 3:

The mean per cent undissolved (±s.d.) of the four oestradiol fractions.

KEY: ● $F_I$ suspension, ▲ $F_{II}$ suspension, ★ $F_{III}$ suspension and ○ Standard suspension.

fraction) and Standard, which show almost identical rates of release.

There seems to be a tendency for the Standard to dissolve more rapidly than $F_{II}$ in the beginning, which is in agreement with the fact that the Standard fraction contains more small particles than $F_{II}$. Later in the release, the quantity of $F_{II}$ in solution exceeded that of the Standard, which is in comformity with the size distributions.

In vivo studies

Table 1 shows the area under the oestradiol plasma concentration curves corrected for zero values calculated by the trapezoidal rule at 0-0.25, 0-0.5, 0-1, 0-8 and 0-72 hours.

The areas 0-8 h show statistically different ($p < 0.01$) amounts of oestradiol absorbed from the four suspensions, i.e. the amount of suspension $F_I$ absorbed was higher than that of suspension $F_{II}$ and Standard, which in turn was absorbed in a amount larger than suspension $F_{III}$. In contrast there was only a slightly significant difference ($p < 0.05$) between the preparations in the total amount of oestradiol absorbed during the period corresponding to the area 0-72 hours.

## DISCUSSION

A correlation was demonstrated between the particle-size data on the starting material and the *in vitro* release data from both methods. The data were plotted on semilog paper with $\sigma_g \times d_s$ on the abscissa and per cent dissolved in vitro on the ordinate. Linear regression analyses were performed on the data on the 15 and 30 min. release, and a correlation coefficient

TABLE 1

AREA UNDER THE OESTRADIOL PLASMA CONCENTRATIONS CURVES

The mean areas (AUC) $\pm$s.d. calculated from the oestradiol plasma concentration curves for the four suspensions $F_I$, $F_{II}$, $F_{III}$ and Standard, based on samples from five patients

| | Area pg/ml x h | | | |
|---|---|---|---|---|
| | Standard | $F_I$ | $F_{II}$ | $F_{III}$ |
| AUC 0-0.25 h | 11 $\pm$ 6 | 54 $\pm$ 31 | 11 $\pm$ 4 | 3 $\pm$ 2 |
| AUC 0-0.5 h | 36 $\pm$ 18 | 167 $\pm$ 102 | 36 $\pm$ 12 | 8 $\pm$ 6 |
| AUC 0-1 h | 92 $\pm$ 53 | 363 $\pm$ 230 | 94 $\pm$ 40 | 17 $\pm$ 16 |
| AUC 0-8 h | 753 $\pm$ 369 | 1485 $\pm$ 717 | 985 $\pm$ 316 | 322 $\pm$ 279 |
| AUC 0-72 h | 2379 $\pm$ 1131 | 3902 $\pm$ 1277 | 3355 $\pm$ 1552 | 1862 $\pm$ 1558 |

of -0.983 was obtained in both cases. (See Figure 4).

An in vivo/in vitro correlation can be demonstrated by comparing the values for AUC and % dissolved oestradiol from both in vitro dissolution methods.

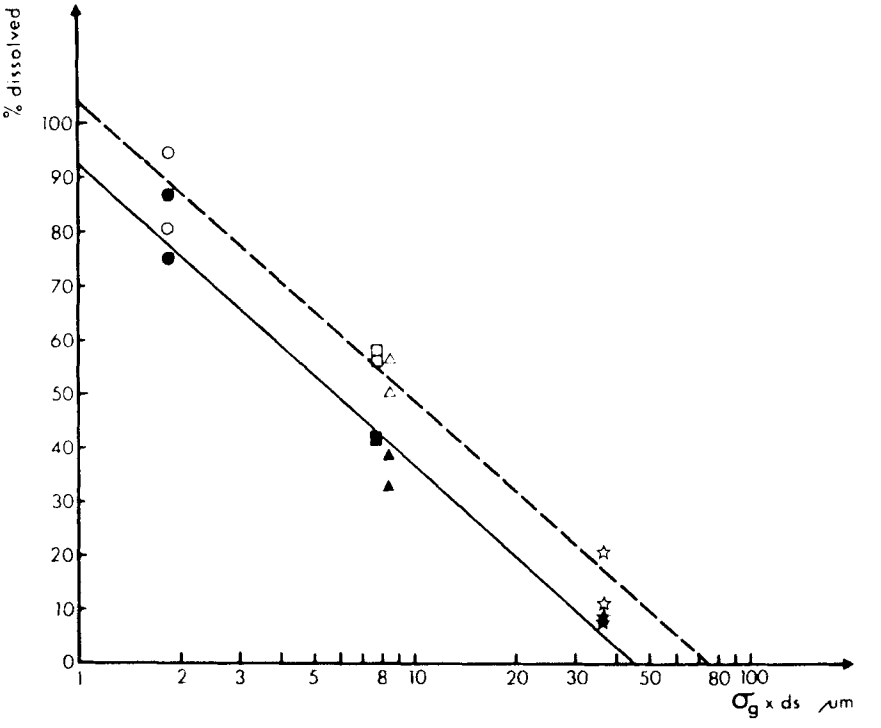


FIGURE 4:

Correlation between the particle-size parameter $\sigma_g$ - $d_s$ per cent dissolved from both dissolution methods.

KEY: —— 15 min, ● $F_I$ fraction, ▲ $F_{II}$ fraction, ★ $F_{III}$ fraction, ■ Standard fraction. ---- 30 min, ○ $F_I$ fraction, △ $F_{II}$ fraction, ☆ $F_{III}$ fraction, □ Standard fraction. $Slope_{15\ min}$ -56.49, $slope_{30\ min}$ -55.62 and correlation coefficient -0.983 for both.

TABLE 2

IN VIVO/IN VITRO CORRELATION

Correlation coefficients from linear regression of AUC and per cent dissolved oestradiol for the four suspensions. The table anly shows the correlation resulting in correlation coefficients > 0.950.

| In vitro method | Sampling time (h) in vitro | Time (h) for the areas (AUC) | Correlation coefficient |
|---|---|---|---|
| Paddle | 0.25 | 0-0.25 | 0.955 |
| | 0.25 | 0.25-0.5 | 0.963 |
| | 0.25 | 0.25-1 | 0.974 |
| | 0.25 | 0-0.5 | 0.960 |
| | 0.25 | 0.5-1 | 0.979 |
| | 0.25 | 0-1 | 0.972 |
| | 0.50 | 0-8 | 0.956 |
| Resistance particle counter | 0.25 | 0.5-1 | 0.957 |
| | 0.25 | 0-8 | 0.970 |
| | 0.25 | 0.25-8 | 0.968 |
| | 0.25 | 0.5-8 | 0.960 |
| | 0.5 | 0-8 | 0.968 |
| | 0.5 | 0.5-8 | 0.971 |
| | 0.5 | 1-8 | 0.965 |

Linear regression analyses for the four oestradiol suspensions were performed by comparison of per cent dissolved oestradiol at the different in vitro sampling times and the calculated areas from 0-0.25, 0-0.5, 0-1, 0-8, 0-72 hours and possible segments obtained by subtraction. Table 2 shows the correlation resulting in correlation coefficients $> 0.950$.

The best correlation is obtained bu comparing initial absorption to in vitro release within the first sampling times 15 and 30 min. Therefore, the in vitro dissolution test could be limited to cover a time period of 30 min., provided sampling within the first 15 min. is more extensive, which might lead to an even better correlation than the one obtained in this study.

The two different in vitro dissolution methods used proved to be equivalent in regard to profiles and reproducibility; the natural choice would be the most simple and less time-consuming of the two, which in our opinion is the resistance particle counter method.

In view of these results, other particle-size distributions and the influence of different formulation factors should be the subject for further studies.

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