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Quantification of depletion in solution-type topical preparations *in vivo*

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Synopsis

To distinguish between thermodynamic, penetration-enhancing, and permeant depletion effects of solution-type transdermal preparations, two sets of human *in vivo* permeant penetration data obtained under finite-dose and infinite-dose conditions, respectively, were compared. The measurement of the pharmacodynamic response of a permeant or the determination of permeant penetration rates under finite-dose conditions includes all three types of vehicle effects, whereas under infinite-dose conditions permeant depletion does not play a significant role. Four lipophilic liquids (caprylic/capric triglyceride, isopropyl myristate, light mineral oil, and dimethicone 100) were used as vehicles. The reciprocal of the time of onset of an erythema (1/LT) and the duration of the erythema D induced by the model compound methyl nicotinate served as response parameters. Steady-state penetration rate measurements were made with a recently developed glass chamber system. Bioavailability factors f and enhancement factors EF were determined from both methyl nicotinate penetration rates (infinite-dose conditions) and the horizontal distances between concentration-response curves (finite-dose conditions), with caprylic/capric triglyceride as the standard vehicle. Permeant depletion was quantified by dividing the enhancement factors determined under infinite-dose conditions by those under finite-dose conditions. These ratios were called depletion factors DF. Significant methyl nicotinate depletion was observed with both response parameters. However, the most accurate bioavailability factors may be obtained with the response parameter 1/LT, especially if they are determined from the horizontal distances between the curves in the high-response region where parallelism is given.

INTRODUCTION

With solution-type permeant preparations applied to the skin, three types of penetration-influencing effects may be observed: (a) thermodynamic effects resulting from different permeant solubilities in the vehicles, (b) penetration-enhancing effects caused by an interaction of the vehicle with the barrier stratum corneum, and (c) permeant depletion in the vehicle in the case of finite-dose conditions.

Permeant depletion may result from a high thermodynamic activity of the permeant in the vehicle and/or from pronounced penetration enhancement caused by vehicle components or by the vehicle itself. The extent of this effect depends on the thickness of the

applied ointment layer. Because ointments are usually applied to the skin as thin films, decreased permeant penetration rates always have to be taken into consideration (1). Permeant depletion may lead to a pronounced reduction of the permeant-induced response and thus have a major impact on the quantification of vehicle effects using pharmacodynamic response data.

More or less sophisticated mathematical models have been developed to describe permeant depletion (1–7). The relationship between the applied drug dose and the pharmacodynamic response using the response parameters' duration and time until onset of the effect has been described mathematically by Levy (8). It was found that under certain conditions a linear relationship between drug dose and response may be obtained.

The objective of this study was to quantify vehicle effects, in particular permeant depletion in the vehicle, using data from two recently conducted human *in vivo* penetration studies that were performed under finite-dose and infinite-dose conditions, respectively (9,10).

MATERIALS AND METHODS

Both *in vivo* studies included the following lipophilic vehicles: dimethicone (DIM; Baysilone M 100®, Bayer AG, Leverkusen, Germany), light mineral oil (MO; Parafluid Mineralölgesellschaft, Hamburg, Germany), isopropyl myristate (IPM: Henkel KGaA, Düsseldorf, Germany), and caprylic/capric triglyceride (CCT; Hüls Troisdorf AG, Troisdorf, Germany). CCT was chosen as the standard because it was expected to show the least pronounced depletion due to the high solubility of the model compound in this vehicle and its inert behavior with regard to skin penetration enhancement (10). The rubefacient methyl nicotinate (MN; Janssen Chimica, Beerse, Belgium) was used as a model compound.

The measurements of the response and the determination of the MN penetration rate were done with 11 (response parameter duration), 10 (response parameter 1/time of onset) and 12 (penetration rate measurements) healthy volunteers, respectively. All experiments were done under occlusion conditions under the assumptions that the MN preparations do not undergo significant changes in composition and that the influence of boundary layers is negligible (11).

MEASUREMENT OF THE RESPONSE (FINITE DOSE)

Solution-type ointment formulations of the above-mentioned lipophilic liquids were prepared by adding either 10% polyethylene (10 kdaltons) or, in the case of DIM, dimethicone (1,000 kdaltons) as gelling agents. Five ointment preparations, containing different amounts of MN, depending on its solubility, and two placebo formulations were applied to the ventral side of each forearm in a double-blind manner under occlusion conditions as described previously (9). The thickness of the applied ointment films was 50 μm, and the area of application amounted to 3.14 cm^2. For every applied preparation, the time of onset of the erythema LT and the duration of the erythema D were determined visually (9). With the resulting data, concentration-response curves were plotted, which allowed the determination of the relative bioavailability as the horizontal distances between the standard curve and the test curves.

MEASUREMENT OF MN PENETRATION (INFINITE DOSE)

For the penetration study a recently developed glass chamber system was used (11). Briefly, two glass cells were fastened to both upper arms of each subject, allowing the examination of four MN preparations at the same time under occlusion conditions. The glass cells were filled with MN solutions of equal permeant activity, emptied after one-hour time periods, and refilled with the initial MN solutions. The MN concentration of the donor phase samples was measured spectrophotometrically. Because the concentration decrease in each one-hour time interval was $\leq 10\%$, zero-order kinetics were assumed. MN disappearance rates were calculated from the concentration differences between the initial solution and the samples obtained after every hour.

CALCULATIONS AND DATA TREATMENT

Bioavailability factors f and f_b. Using Fick's first law of diffusion, bioavailability factors f may be obtained by calculating the ratios of the first order penetration rate constants of the test vehicles R_T and the standard vehicle R_{ST}:

$$f = R_T/R_{ST} \qquad \text{(Eq. 1a)}$$

where the penetration rate constant R is defined as follows:

$$R = D_B \cdot A \cdot PC_{B/V}/(d_B \cdot V) \qquad \text{(Eq. 1b)}$$

where D_B is the diffusion coefficient of the permeant in the barrier stratum corneum, A is the application area, $PC_{B/V}$ is the stratum corneum/vehicle partition coefficient of the permeant, d_B is the thickness of the stratum corneum, and V is the volume of the applied preparation.

The ratio V/A is an expression of the thickness h of the ointment layer. In the case of penetration rate data, bioavailability factors may be determined as the ratio of the penetration rate constants R obtained with the test vehicles and with the standard vehicle. Penetration rate constants are calculated as the quotient of the steady-state penetration rate and the permeant amount in the vehicle.

From the horizontal distance between the parallel portions of the dose-response curves of a standard preparation (ST) and a test preparation (T) at a certain response level Resp%, the bioavailability factor f is determined as follows (Figure 1):

$$\log f = \log \text{dose}_{\text{Resp\%ST}} - \log \text{dose}_{\text{Resp\%T}} \qquad \text{(Eq. 2a)}$$

$$f = \text{dose}_{\text{Resp\%ST}}/\text{dose}_{\text{Resp\%T}} \qquad \text{(Eq. 2b)}$$

In practice, the shape of the dose-response curves is sigmoidal for the response parameter 1/LT. A plateau is reached as soon as the permeant solubility limit in the vehicle is exceeded (Figure 2a). This plateau may be elevated under the influence of penetration enhancers. With the response parameter D, a sigmoidal shape of the curves cannot be expected because the pharmacodynamic effect will last as long as the amount of dissolved permeant in the vehicle, and thus the penetration rate is high enough, no matter if permeant solutions or suspensions are applied. In addition, the more R decreases, the more pronounced the reservoir function of the applied preparation will become and the higher the gradient of the curve will be, which at a certain dose level even leads to an intersection of the test and the standard curve (Figure 2b). Therefore, the determination

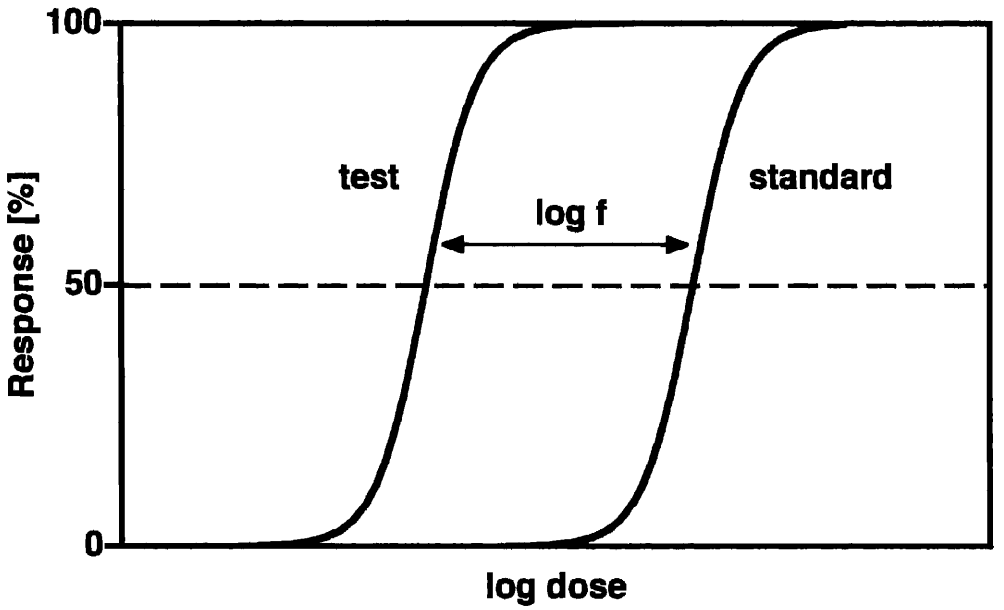

Figure 1. Determination of the bioavailability factor f from the dose/response curves of a test and a standard preparation.

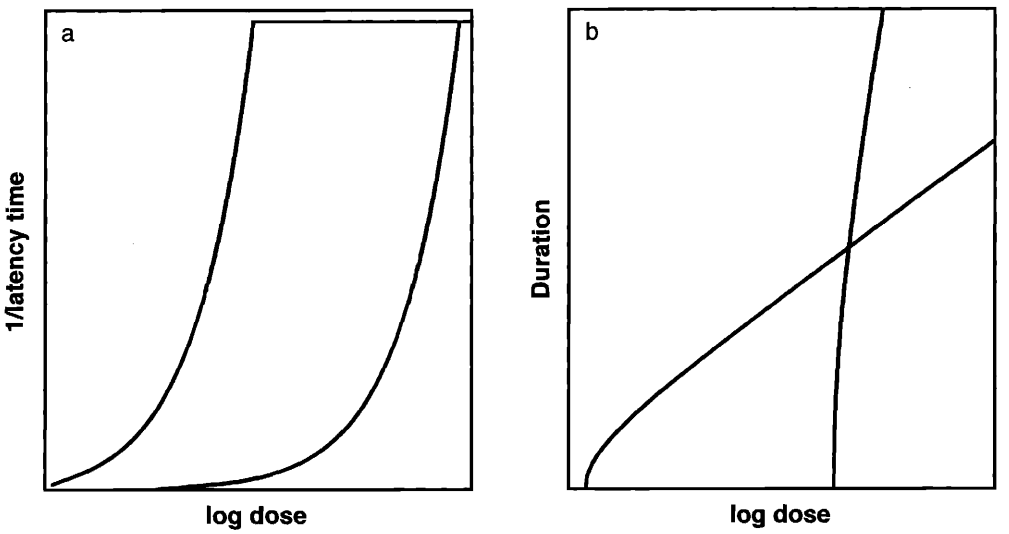

Figure 2. Typical dose-response curves obtained with the response parameters 1/time until onset (a) and duration (b) of an effect. The curves resulting from two different R values were simulated with the Bateman equation assuming an open one-compartment model and a negligible lag time.

of bioavailability factors is more accurate in the lower part of the curves, where the duration of the effect is short (12).

It has to be mentioned that in the case of dose-response curves, f also depends on the volume of the applied vehicles. The use of concentration-response curves mathematically eliminates the influence on f of the preparation volume and, assuming equal areas of

application for test and standard preparations, also the thickness of the preparation. After elimination of the ointment film thickness, the resulting bioavailability factors are called f_h:

$$\log f_h = \log c_{Resp\%ST} - \log c_{Resp\%T} \quad \text{(Eq. 3a)}$$

$$f_h = c_{Resp\%ST}/c_{Resp\%T} \quad \text{(Eq. 3b)}$$

$$f_h = R_T \cdot h_T/(R_{ST} \cdot h_{ST}) = P_{B_T}/P_{B_{ST}} \quad \text{(Eq. 3c)}$$

From measurements of the permeant, penetration rate bioavailability factors may therefore be determined as the ratio of the permeant permeabilities $P_B = D_B \cdot PC_{B/V}/d_B$ obtained with the test vehicles and the standard vehicle. The permeant permeability P_B is calculated as the steady-state permeant penetration rate multiplied by the preparation thickness (=V/A) and divided by the permeant amount in the vehicle.

Enhancement factor EF. Enhancement factors may be calculated by dividing the bioavailability factors f_h by the relative effective activity coefficient $\gamma_{T/ST}$ (Eq. 4a), which is defined as the ratio of the permeant partition coefficients ST/reference phase and T/reference phase (13). Procedures to determine these partition coefficients have been described (10,13).

$$EF = f_h/\gamma_{T/ST} = f_a \quad \text{(Eq. 4a)}$$

In absence of penetration enhancement and permeant depletion, f_h equals $\gamma_{T/ST}$. Enhancement factors EF, which sometimes are called activity-standardized bioavailability factors f_a (14), may be calculated from the horizontal distance between activity-response curves, where the relative permeant activity a is the product of the permeant concentration in the vehicle and $\gamma_{T/ST}$.

$$\log EF = \log a_{Resp\%ST} - \log a_{Resp\%T} \quad \text{(Eq. 4b)}$$

$$EF = a_{Resp\%ST}/a_{Resp\%T} \quad \text{(Eq. 4c)}$$

With activity-response curves, not only the influence of the preparation thickness h but also the influence of the permeant solubility in the vehicle CsB on the bioavailability factor f is mathematically eliminated. Provided that the thickness of the stratum corneum is not affected by the ointment bases, enhancement factors are only dependent on the permeant diffusion coefficient D_B and the permeant solubility in the barrier CsB and may therefore be written as

$$EF = D_{B_T} \cdot CsB_T/(D_{B_{ST}} \cdot CsB_{ST}) \quad \text{(Eq. 4d)}$$

In the case of the penetration rate data, EF values may also be calculated as the ratio of the steady-state permeant penetration rates from a test vehicle ($ssPRa_T$) and the standard vehicle ($ssPRa_{ST}$), provided that the permeant activity a is the same in all vehicles and again assuming equal application areas for test and standard preparations.

$$EF = ssPRa_T/ssPRa_{ST} \quad \text{(Eq. 4e)}$$

Equal permeant activities in the vehicles may be obtained if the initial permeant concentration in the standard vehicle is divided by the $\gamma_{T/ST}$ value of the test vehicles.

The direct influence of the factors A, V, and CsV on the bioavailability factor f may be eliminated by the above-mentioned mathematical procedures. However, it has to be taken into consideration that the contribution of all of these factors to permeant depletion cannot be eliminated by these calculations, a fact which may lead to a false

estimation of the relative bioavailability as a result of an insufficient parallelism of the dose-response curves resp. a reduction in the penetration rate.

If an enhancement effect is mainly caused by an increase of the permeant diffusion coefficient in the stratum corneum, it has to be considered that the lag time, which depends not only on the thickness of the barrier but also on the permeant diffusion coefficient in the barrier (15), may also influence the shape of the dose-, concentration-, or activity-response curves, particularly those obtained with the response parameter 1/LT: the resulting curves do not run parallel to each other and different plateau values may be reached. In this case a correct estimation of the bioavailability factor from the distance of the curves is impossible. However, it has been found that pronounced penetration enhancement is mainly caused by an increase of the permeant solubility in the barrier C_{SB} rather than by an increase of the permeant diffusion coefficient D_B (16).

Depletion factor DF. Any increase of R leads to a more or less pronounced permeant depletion. This phenomenon is not described by the above-mentioned equations. With the data of both *in vivo* studies, permeant depletion, which usually occurs under finite-dose conditions and which manifests itself in a significant decrease of the permeant penetration rate and thus in an insufficient parallelism of the dose-, concentration-, or activity-response curves, can be quantified. In order to do so, a so-called depletion factor DF is introduced and can be calculated from the infinite-dose (inf) and finite-dose (fin) enhancement factors EF as follows:

$$DF = EF_{inf}/EF_{fin} \qquad \text{(Eq. 5)}$$

Depending on which vehicle is chosen as standard, these depletion factors can reach values greater or smaller than unity. A standard vehicle that shows pronounced penetration-enhancing properties leads to values ≤ 1, whereas an inert standard vehicle leads to values ≥ 1. It again has to be mentioned that not only D_B and C_{SB} as described by the enhancement factor but every single factor included in R contributes to the extent of permeant depletion.

RESULTS AND DISCUSSION

The bioavailability factors f_h resulting from the *in vivo* studies are shown in Figure 3. As the extent of permeant input can be assumed to be 100% with all three methods, any differences in f_h are attributable to differences in the rate of permeant input. In absence of penetration enhancement and depletion effects, all data points should be located on the theoretical straight line described by the equation $f_h = \gamma_{T/ST}$. Deviations from the straight line may be interpreted as follows: higher values as in the case of IPM result from penetration enhancement, whereas lower values indicate MN depletion of the preparations, which is particularly obvious in the case of the response parameter duration of the erythema. Permeant depletion manifests itself in an insufficient parallelism of the concentration-response curves, which may lead to an underestimation of f_h if the standard vehicle is inert with regard to penetration enhancement and shows the highest MN solubility as compared to the test vehicles. As in the case of the time of onset, the duration of the effect, i.e., the time period during which the MN concentration at the receptor site is above the threshold concentration required for an effect to become obvious, depends on the permeant penetration rate and thus on R and the applied permeant dose. If the penetration rate decreases rapidly over time as in the case of high

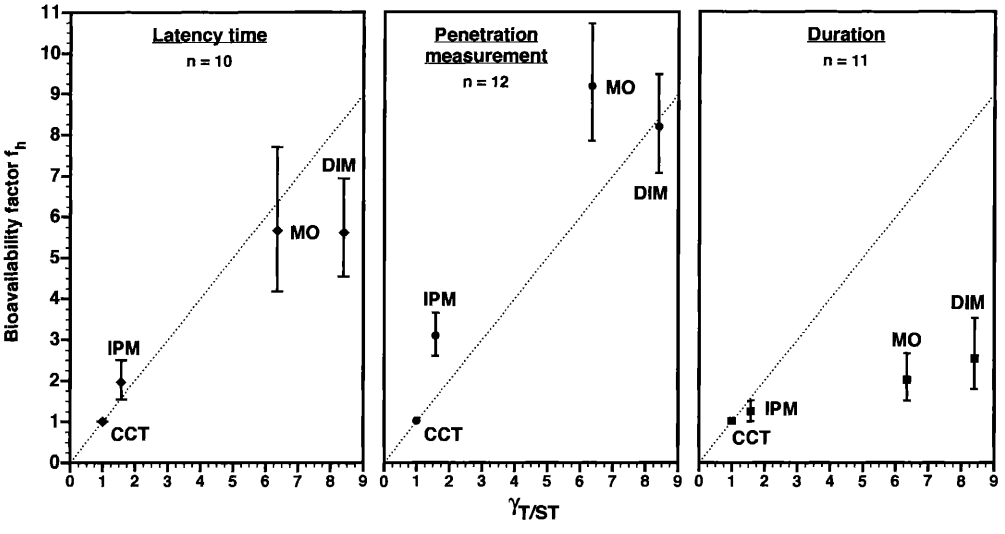

Figure 3. Bioavailability factors f_h as a function of the relative effective activity coefficient $\gamma_{T/ST}$. The dotted line represents the function $f_h = \gamma_{T/ST}$. Error bars are 95% confidence intervals.

R values, this time period, i.e., the duration of the effect, is reduced. As mentioned earlier, the most accurate bioavailability factors may be determined from the low-dose range of the curves where the duration of the effect is rather short (Figure 2b). However, even in the low-dose range of the curves, the theoretical f values will never be reached, leading to the conclusion that the duration of the effect is an unsuitable parameter of response. The response parameter 1/LT is only marginally influenced by decreased penetration rates because the onset of the erythema occurs within minutes after application of the ointment. Thus, permeant depletion will only become obvious if the permeant penetration rate is markedly reduced. High penetration rate constants may lead to an underestimation of the response values in the low response region of the concentration-response curves, which is the reason for the deviation of the f_h values from the theoretical or the infinite-dose values as shown with DIM (Figure 3). With increasing permeant concentration in the vehicle and thus decreasing latency time, this deviation becomes less evident and even disappears at high permeant concentrations (Figure 2a). Theoretical or infinite-dose values can only be obtained from the upper parts of the curves right below the plateau where parallelism is given (12).

Generally, the higher the horizontal distance between the dose-response curves of a test and a standard preparation, i.e., the higher the R_T/R_{ST} ratio, the more pronounced permeant depletion will become, which manifests itself in underestimated bioavailability factors. It is therefore preferable to determine the horizontal distances between dose-response curves instead of concentration- or activity-response curves in order to get a better impression of the extent of permeant depletion.

The greater bioavailability factors resulting from the infinite-dose penetration rate data are not only a result of the minimized permeant depletion but are also due to penetration enhancement in the case of IPM and MO. From concentration-response curves, no specific vehicle effects can be detected with MO because of an interference of penetration enhancement with permeant depletion (Figure 3). For this reason, no penetration-enhancing effect could be found with MO in a recent study (9).

Enhancement factors obtained with the different vehicles under infinite- and finite-dose conditions, respectively, are shown in Figure 4. In the past, enhancement factors describing only the specific effects of vehicles on the properties of the barrier stratum corneum were determined by several investigators (17–19). These factors correspond to the enhancement ratio introduced by Goodman and Barry (20) and to the so-called activity-standardized bioavailability factor f_a, which can be determined from activity-response curves (14).

The data presented in Figure 4 again show very pronounced MN depletion for MO and DIM, which is less obvious with the parameter 1/LT. In contrast to the pharmacodynamic response data, the results of the penetration rate data clearly show that IPM and MO act as penetration enhancers. Although MO is considered to be an inert vehicle, it has been shown to fluidize the lipid bilayers of the stratum corneum to some extent, possibly a result of its branched structure (21).

Depletion factors calculated as the ratio of the enhancement factors obtained under infinite-dose conditions to those obtained under finite-dose conditions are shown in Figure 5. From the data it is obvious that with both response parameters a statistically significant MN depletion may be observed. From the fact that MN depletion is significant with the response parameter 1/LT, one may conclude that the determination of the relative bioavailability was not done in the high-response region of the concentration-response curves. MN depletion seems to be more pronounced with MO than with DIM. This is due to the fact that MO causes penetration enhancement in addition to the high MN activity in this vehicle. It behaves like a vehicle with a thermodynamic activity of MN, even higher than that with the vehicle DIM, which does not cause penetration enhancement of the model compound. This indicates that, among others, a high thermodynamic activity of the permeant and penetration-enhancing properties of the vehicle may lead to permeant depletion in the vehicle.

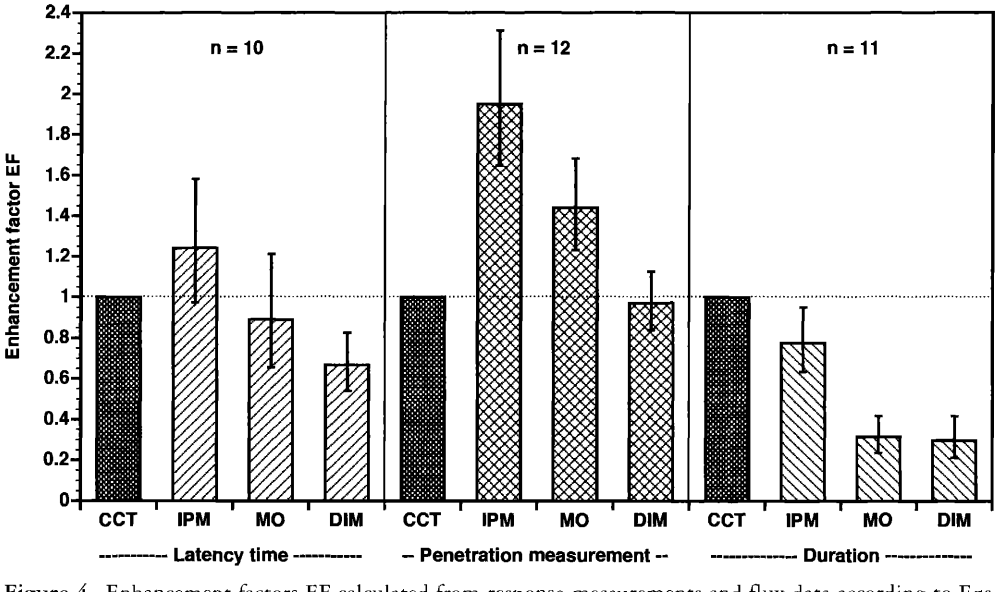

Figure 4. Enhancement factors EF calculated from response measurements and flux data according to Eqs. 4a/e. Error bars are 95% confidence intervals.

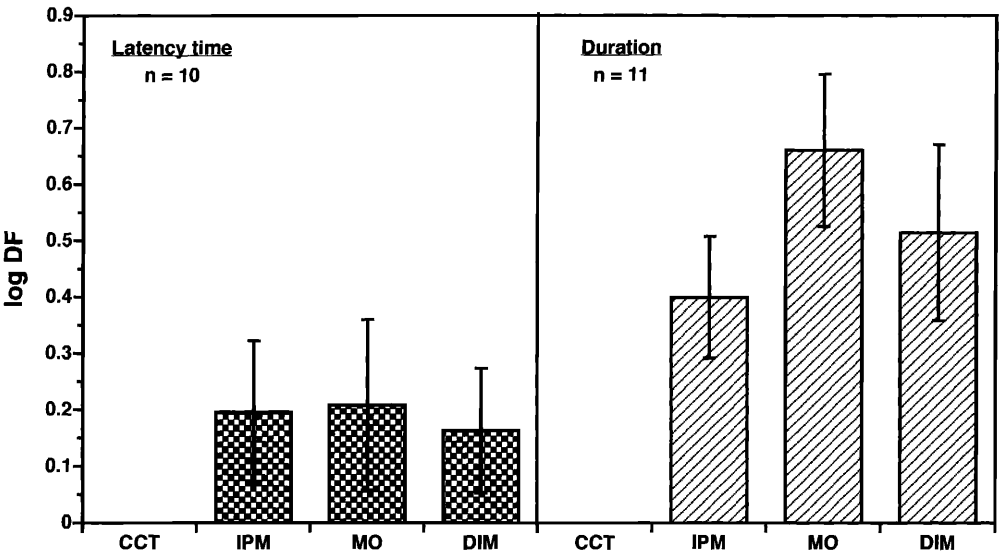

Figure 5. Depletion factors DF calculated for the response 1/LT and D according to Eq. 5. Error bars are 95% confidence intervals.

From the presented results it may be concluded that any increase of the penetration rate constant may lead to permeant depletion. The mathematical elimination of parameters such as the thickness of the applied ointment formulation or the permeant solubility in the vehicle in order to differentiate between vehicle effects does not eliminate their influence on permeant depletion. Because of the insufficient parallelism of the dose-response curves, the duration of the erythema is an unsuitable parameter for the evaluation of thermodynamic or specific vehicle effects. In the case of the response parameter 1/latency time, correct estimations of the relative bioavailability can be expected only in the high-response region. Permeant depletion in the vehicle has to be considered particularly if the permeant solubility in the vehicle is low and/or in the case of vehicles with penetration-enhancing properties. It can be avoided either by application of suspension-type preparations or by using vehicles with high dissolving capacities.

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