D 20485 E

Die Pharmazie

An International Journal of Pharmaceutical Sciences

3 48. Jahrgang
März 1993

Aus dem Inhalt

GOVI

**Govi-Verlag
Pharmazeutischer Verlag GmbH
Frankfurt am Main**

PHARAT 48 (3) 161–240 (1993)
ISSN 0031-7144 · Pharmazie · Frankfurt/Main · 48 (1993) 3 · S. 161–240

Lehrstuhl für Biopharmazie[1] der Universität Halle und Institut für Pharmazeutische Technologie und Biopharmazie[2] der Universität Heidelberg

Study of the in vitro penetration of the topical glucocorticoid betamethasone-17-valerate from solution-type gels into a multilayer membrane system

BARBARA BENDAS[1], A. GÖPFERICH[2], G. LEE[2] and R. NEUBERT[1]

The *in vitro* transport of betamethasone-17-valerate (**1**) into a multilayer membrane system has been investigated. Subsaturated formulations of **1** were studied as formed by mixing appropriate propylene glycol/water cosolvent systems. The AUC (drug concentration in acce ptor membrane as a function of time) and the diffusivity of the drug in the vehicle were used to evaluate the results of the *in vitro* transport. The importance and relationship between solubility, partition coefficient, and diffusivity for the process of *in vitro* penetration of **1** are discussed.

Untersuchungen zur in vitro-Penetration von Betamethason-17-valerat aus Lösungsgelen in ein Mehrschichtmembransystem

Gegenstand der Untersuchungen ist der in vitro-Transport von Betamethason-17-valerat (**1**) aus Lösungsgelen in ein Mehrschichtmembranmodell. Als Hydrogele kamen Systeme mit unterschiedlichen Propylenglycol/Wasser-Mischungen zur Anwendung. Zur Auswertung der Ergebnisse wurden die AUC (Arzneistoffkonzentration in der Akzeptormembran als Funktion der Zeit) und der Diffusionskoeffizienz des Arzneistoffes im Vehikel herangezogen. Die Bedeutung und der Zusammenhang zwischen Löslichkeit, Verteilungskoeffizient und Diffusionskoeffizient für die in vitro-Penetration von **1** werden diskutiert.

1. Introduction

The importance of the interactions between drug, vehicle, and skin for percutaneous absorption are complex, as can be appreciated from a study of the available literature [1–6]. There are various parameters that affect the mechanisms responsible for specific drug release and penetration. One way to improve the dermal absorption of drugs is to manipulate their physico-chemical properties by producing derivatives with increased potential for permeation through the skin barrier. Many investigations have been carried out to produce prodrugs with the aim to increase lipophilicity. This changes related parameters like partition coefficient, solubility and even diffusivity [7–16].

The dermal uptake properties of a drug are also influenced by the choice of the type of vehicle and its ingredients. Thus the interactions of the physico-chemical properties of a drug and vehicle play an important role for the delivery of the drug into the skin from its formulation [17–20]. Furthermore, the components of the vehicle are themselves able to penetrate into the skin. This can result in a decreased diffusional resistance of the stratum corneum [21–23].

In this study, betamethasone-17-valerate (**1**) in the mixed cosolvent system propylene glycol (PG)/water has been used to study solution-type gels as topical vehicles. The influence of PG on the *in vitro* penetration of the drug out of the vehicle into a membrane system has been investigated. The ratio of the **1** concentration in solution (C_v) to its saturated solubility in the gel (C_s) was maintained constant, with the drug only being present in the dissolved state.

2. Investigations, results and discussion

The *in vitro* penetration experiments were run using solution-type formulations of **1** in simple hydrogels with varying content of cosolvent. To classify the lipophilicity of **1** its saturated solubility in the acceptor lipid dodecanol (DD), in propylene glycol (PG) and in water was determined (Table 1). It is slightly soluble in water, but shows a high solubility in DD. The percentage of dissolved **1** in water increases with increasing PG content (Fig. 1). If the ratio of C_v to C_s is kept constant for all systems investigated, then the same solubility conditions are established. From previous results [31] one can expect that for fixed thermodynamic activity, the amount penetrated into the membrane will be constant up to a certain value. Above this, the release of **1** will be controlled by partition behaviour.

Table 1 Solubility of 1 in DD, PG and water as solvent systems (mean value ± SD, n = 4)

	DD	PG	Water
Solubility [mg/ml]	12.5 ± 1.0	3.0 ± 0.01	0.012 ± 0.008

In this study, the AUC was used as a parameter to estimate the amount and rate of *in vitro* penetration of **1** from each gel as a function of time and PG-content (Fig. 2).

Consideration of the cumulative amount of **1** transported in dependence on the time shows that with PG content above 50% the amount penetrated after 200 min decreases (Fig. 2). When the AUC is expressed as a function of PG content (see Table 2) it can be estimated that the *in vitro* penetration is independent of PG content up to 40%. The slight increase in AUC values in the range of 50 and 60% PG are caused by higher amounts of **1** being transported within the first 15 min (Fig. 3.). This increased penetration rate is caused by convective transport. It has been found that PG is able to penetrate

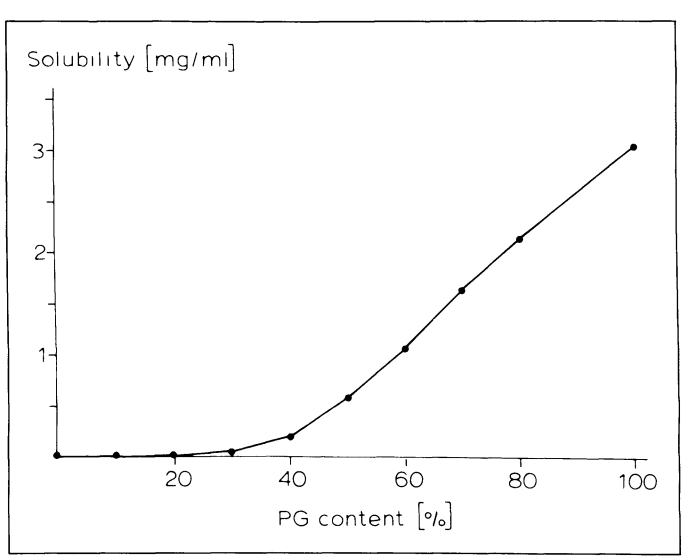

Fig. 1. Solubility of **1** in PG/water mixtures

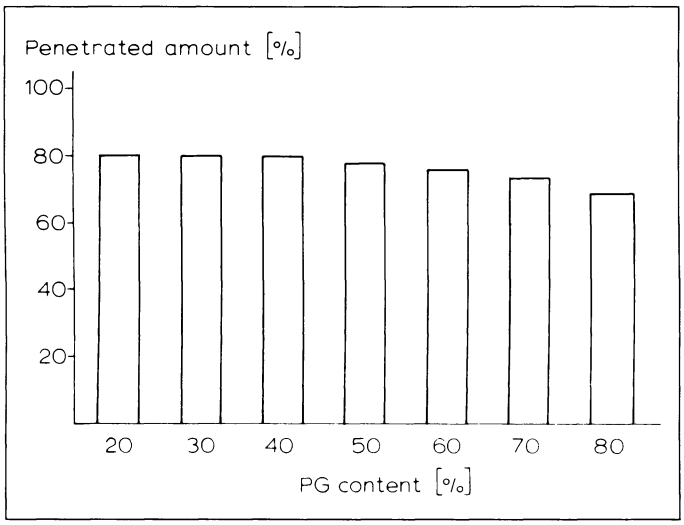

Fig. 2. *In vitro* penetration of **1** from solution-type gels as function of PG

considerably in the acceptor membranes [32]. Drug molecules dissolved in the cosolvent are, therefore, transported with the cosolvent flow into the acceptor. With increasing PG content in the gels, the amount of **1** penetrated into the acceptor decreases, although the extent of convection would be expected to increase.

The study of the partition behaviour of **1** between vehicle and acceptor indicates that the ability of **1** to distribute into the acceptor decreases with increasing PG content of the donor medium (Fig. 4). By comparing the solubility and partition behaviour as functions of PG content, it is evident that these two contrary processes result in an optimal cosolvent concentration for the *in vitro* penetration of **1** [33–35]. The partition coefficient will be the most important parameter, provided the concentration of drug is in a constant relation to its saturated solubility and it other factors influencing the drug release, such as change in viscosity of the vehicle, can be neglected. The decrease in partition coefficient seen with higher cosolvent concentration in the gels arises from a higher affinity of the drug for the vehicle, consequently lower uptake rates of **1** are measured.

The diffusion coefficients for the **1** in the vehicles lie in the range of 10^{-7} cm^2/s (Fig. 5) as is typically found for isotropic hydrogels. They are independent of the PG content up to 40%, At higher PG contents, D decreases slightly but remains within the same order of magnitude. The higher affinity of the drug for the formulation at these large PG contents manifests itself in lower values for K. In accordance with Fick's First law, these two changes result in a decrease in the amount of 1 penetrated.

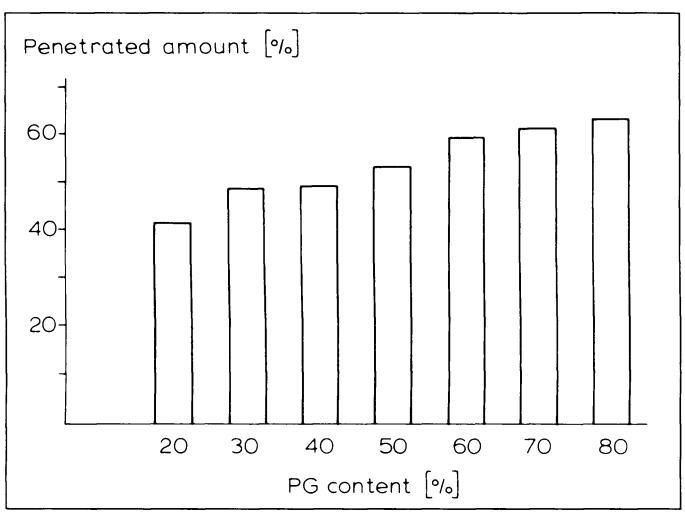

Fig. 3. Penetrated amount of **1** in dependence of PG content within 200 min

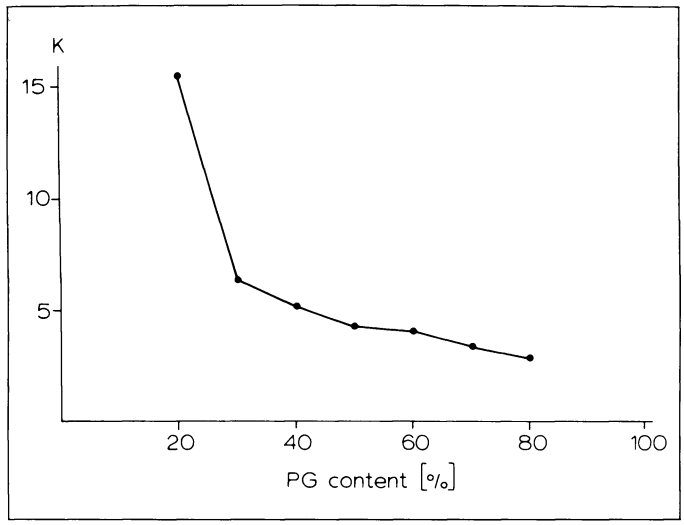

Fig. 4. Penetrated amount of **1** in dependence of PG content in 15 min

We conclude that both AUC and diffusivity can be used to evaluate the *in vitro* penetration of a lipophilic substance from simple hydrogels (Table 2). The calculation of diffusivity allows a quantitative estimation of the importance of the AUC.

Table 2 Comparision of the parameters AUC and diffusivity for evaluating the in vitro penetration of 1 from hydrogels with varrying PG content

PG content [%]	AUC [% · min]	D [cm^2/s]
20	13690	1.105 E-007
30	13639	1.179 E-007
40	13439	1.173 E-007
50	13866	8.204 E-008
60	13734	8.006 E-008
70	11743	6.4865 E-008
80	11597	6.2296 E-008

3. Experimental

3.1. Materials

Betamethasone-17-valerate (**1**) and propylene glycol (PG) were purchased from COM Pharma Handels-GmbH, Hamburg. Chloroform, methanol and collodion (4% w/w) were provided by Laborchemie Apolda. Dodecanol (DD), tetrazolium blue and potassium hydroxide were purchased from Merck, Darmstadt and sodium carboxymethylcellulose from Fluka Feinchemikalien GmbH, Neu-Ulm.

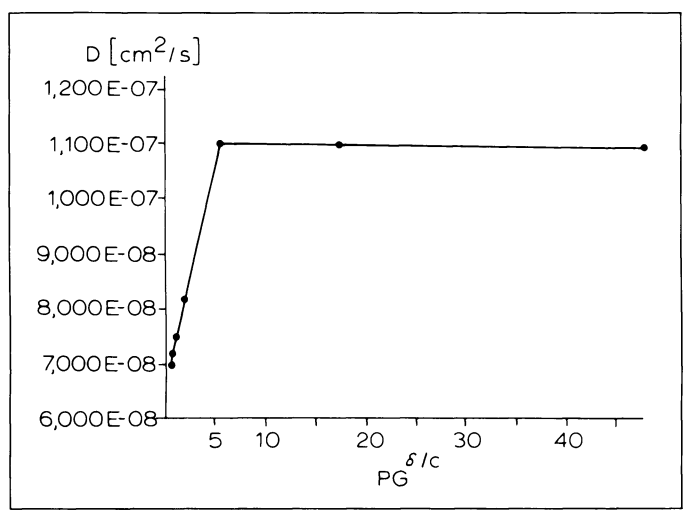

Fig. 5. Partition coefficient of **1** between vehicle and acceptor lipid

3.2. Methods

3.2.1. Solubility of **1** *in PG water mixtures*

A series of PG/water mixtures was prepared from 10 to 80% PG in 10% (w/w) increments. Drug was added to each until a suspension formed, which was then shaken for 24 h at 32 C + 1 C. The samples were centrifuged and the supernatant solutions were assayed for their drug content. The tetrazolium blue method for glucocorticoids was used.

3.2.2. Determination of partition behaviour

To characterize the partition behaviour of **1** between the solution-type gels and the acceptor medium (DD) the penetration experiments were carried out for a finite time (300 min). The drug content in the membranes was then determined, and the partition coefficient (K) calculated according to Nernst:

$$\frac{a_{org}}{a_{aqu}} = K .$$

The following conditions have to be taken into account: 1. non ideal, dilute solutions were used; and 2. the formulation excipients, e.q. PG, could penetrate into the acceptor medium.

3.2.3. Preparation of the gels

A suitable amount of sodium carboxymethylcellulose was mixed with the appropriate amount of PG and the mixture made up with distilled water. The gels were then added to the drug and stirred to a homogenous formulation.

3.2.4. In vitro membrane transport studies

The multilayer membrane system used has been described previously [24, 25]. A defined amount of the formulation (10 mg) was applied to the acceptor system, which was attached in a cell with a fixed application area (4 cm^2). The acceptor system consists of one lipid membrane to maintain approximative sink conditions (acceptor loading after complete absorption of the drug: 1 10%). The experiments were carried out sixfold at 32 C ± 1 C in a temperature-controlled chamber. At selected time intervals the model apparatus was removed from the chamber, the penetration cells were disassembled, the formulation remaining on the exposed surface was removed, and the membranes assayed for their content of **1**.

3.2.5. Extraction of the drug

The separated membranes were extracted with 2 ml for each sample by shaking for 30 min.

3.2.6. Analytical method

The content of **1** in the various membranes of the model was assayed indirectly by measuring absorbance after reduction of tetrazolium blue through the oxidation of the ketol structure of the glucocorticoid in alkaline solution. The absorbance of the formazan compound obtained was measured at 525 nm.

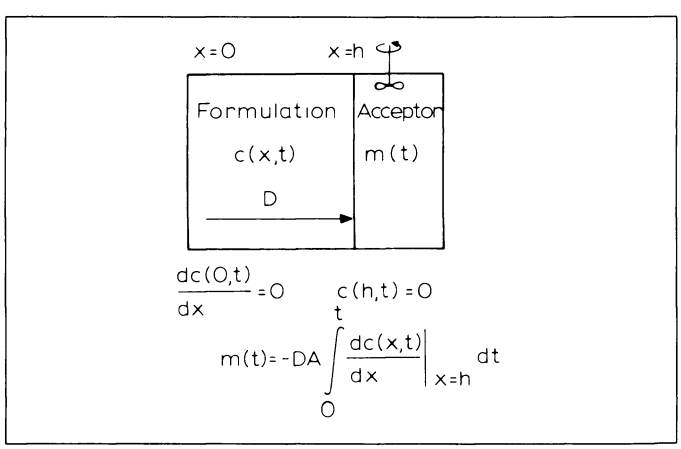

Fig. 6. Sink model for describing the diffusional release of a drug from thin film into adjacent acceptor

3.2.7. Analysis of data

The results were expressed as plots of **1** concentration in the acceptor membrane *versus* time. The statistical moments of each curve were calculated according to Brockmeier [26, 27]. According to the literature, the AUC can be used for characterizing the extent of drug penetration into systems such as excised human skin and the membranes used here [28, 29].
Additionally, the diffusivity of the drug in each formulation was calculated. Theoretical values for the time-dependent mass of drug in the acceptor membrane were fitted by the Nelder-Mead method to the corresponding experimentally determined values. The calculation was based on a finite-difference method [30] to approximate numerically the diffusion equation for the linear movement of a drug with constant diffusity, D, through a finite plane sheet: $Dc(x, t)_{xx} - c(x, t)_t = 0$, where c(x, t) = concentration in formulation, x = space coordinate, and t = time. The sink model used is illustrated in Fig. 6 and describes the diffusional release of the drug from a thin film of formulation into an adjacent acceptor membrane. The latter is taken to act as a sink, resulting in: c(h, t) = 0. The flux of drug entering the acceptor membrane, J, is equal to that leaving the formulation, i.e. $J = dm(x, t)/Adt|_{x=h}$.

References

1 Katz, M.; Poulsen, B. J.: J. Soc. Cosmet. **23**, 565 (1972)
2 Idson, B.: J. Pharm. Sci. **64**, 901 (1975)
3 Lippold, B. C.: Acta Pharm. Technol. **27**, 1 (1981)
4 Horsch, W.: Pharmazie **39**, 598 (1984)
5 Ziegenmeyer, J.: Pharm. unserer Zeit **15**, 129 (1986)
6 Stüttgen, G.: Die Haut als Transportorgan für Arzneistoffe, S. 1, Steinkopff Verlag, Darmstadt 1990
7 Landmann, L.: Pharm. unserer Zeit **20**, 155 (1991)
8 Flynn, G. L.; Yalkowsky, S. H.: J. Pharm. Sci. **61**, 839 (1972)
9 Cooper, E. R.: J. Controlled Release **1**, 153 (1984)
10 Guy, R. H.; Carlström, E. M.; Bucks, D. A. W.; Hinz, R. S.: J. Pharm. Sci. **75**, 968 (1986)
11 Young-Harvey, J. A.; Rae, I. D.; Pitman, I. H.: Int. J. Pharm. **30**, 151 (1986)
12 Johansen, M.; Mollgaard, B.; Wotton, P. K.; Larsen, C.; Hollgaard, A.: ibid. **32**, 199 (1986)
13 Waranis, R. P.; Sloan, K. B.: J. Pharm. Sci **76**, 587 (1987)
14 Al-Khamis, K.; Davis, S. S.; Hadgraft, J.: Int. J. Pharm. **40**, 111 (1987)
15 Tojo, K.; Chiang, C. C.; Chien, Y. W.: J. Pharm. Sci. **76**, 123 (1987)
16 Bundgaard, H.; Mork, N.; Hollgaard, A.: Int. J. Pharm. **55**, 91 (1989)
17 Prustrup, J.; Fullerton, A.; Christrup, L.; Bundgaard, H.: ibid. **71**, 105 (1991)
18 Loth, H.: ibid. **68**, 1 (1991)
19 Asche, H.; Botta, L.; Rettig, H.; Weirich, E. G.: Acta Pharm. Helv. **60**, 232 (1985)
20 Sloan, B. K.; Siver, K. G.; Koch, A. M.: J. Pharm. Sci. **75**, 744 (1986)
21 Nannipieri, E.; Carelli, V.; Di Colo, G.; Giorgi, I.; Serafini, M. F.: Int. J. Cosmet. Sci. **12**, 21 (1990)
22 Hadgraft, J.: Pharm. Int. **5**, 252 (1984)
23 Ritschel, W. A.; Sprockel, O. L.: Drugs of today **24**, 613 (1988)
24 Fürst, W.; Neubert, R.; Wildner, R.: Pharmazie **42**, 452 (1987)
25 Neubert, R.; Wohlrab, W.: Acta Pharm. Technol. **36**, 197 (1990)
26 Brockmeier, D.: Arzneim.-Forsch. **31**, 746 (1981)
27 Idem: Acta Pharm. Technol. 32, 164 (1986)
28 Neubert, R.; Bendas, C.; Wohlrab, W.; Gienau, B.; Fürst, W.: Int. J. Pharm. **75**, 89 (1991)
29 Neubert, R.; Bendas, B.; Wohlrab, W.: Eur. J. Pharm. Biopharm. **38**, 11 (1992)
30 Crank, J.; Nicolson, P. A.: Proc. Camb. Phil. Soc. **43**, 50 (1947)
31 Lippold, B. C.: Acta Pharm. Technol. **27**, 1 (1981)
32 Bendas, B.; Neubert, R.: in preparation
33 Patel, A. R.; Vasavada, R. C.: Pharm. Res. **5**, 116 (1988)
34 Poulsen, B. J.; Young, E.; Coquilla, V.; Katz, M.: J. Pharm. sci. **57**, 928 (1968)
35 Ostrenga, J.; Steinmetz, C.; Poulsen, B.: ibid. **60**, 1175 (1971)

Received September 4, 1992

Prof. Dr. Reinhard Neubert
Weinbergweg 15
4010 Halle/Saale