*International Journal of Pharmaceutics*, 40 (1987) 111–118
Elsevier



IJP 01350

# In vitro–in vivo correlations for the percutaneous absorption of salicylates

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(Received 31 March 1987)
(Modified version received 9 June 1987)
(Accepted 11 June 1987)

*Key words*: Percutaneous absorption; Salicylate; Carbopol; Polyethylene glycol; Particle size; Drug release

**Summary**

Release rates of salicylic acid and the methyl, ethyl, phenyl and glycol esters have been determined from a range of topical formulations. These include representative Plastibases, Carbopol 940 and polyethylene glycols. The release rates can be rationalised in terms of the physicochemical properties of the solutes and the bases in which they are dissolved/dispersed. Selected formulations were placed on the shaved skin on the back area of the ear of male lop rabbits. Plasma samples were removed for the subsequent 7 h period and assayed for salicylate. The concentration of salicylate in the plasma was related to the in vitro release rate and hence the physicochemical properties of the diffusant.

## Introduction

Assessing the bioavailability of topical formulations is a complex process since many factors are involved. The active drug firstly has to be released from the preparation and this process alone can involve various stages. The underlying determinants of release will, however, be the thermodynamic activity of the drug and the microviscosity of the medium through which the drug diffuses. In emulsion formulations where two phases exist the drug may also undergo partitioning steps within the preparation. Whether or not these are rate-determining will depend on the nature of the phases and the physicochemical properties of the drug. At the skin surface the drug will partition from the preparation into the primarily lipid-rich environment of the stratum corneum. It will diffuse slowly through this region of the epidermis and then partition from the stratum corneum into the viable tissue. Further diffusion occurs until the drug reaches the dermal vasculature where it is removed into the systemic circulation. Thus, bioavailability and action are dependent on drug release, transfer across the skin and the interaction of the drug with the appropriate target receptor. Bioavailability is usually defined in terms of the systemic levels of the drug. In the experiments described later blood levels have been measured in order to relate formulation effects to bioavailability.

It is comparatively easy to monitor the in vitro release of a drug from a topical formulation although interpreting the results in terms of basic physicochemical parameters is not facile. Measuring in vivo absorption is more complex. Drug

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penetration can be assessed in a limited number of cases by observing a physiological response, e.g. the vasodilatation induced by the nicotinate esters (Stoughton et al., 1960; Fountain et al., 1969) or vasoconstriction caused by the corticosteroids (Barrett et al., 1965; Woodford and Barry, 1982). Many drugs do not produce detectable physiological responses and skin penetration has then been assessed by analysing either the blood or urine for drug. Since the skin is an excellent barrier the amount of drug penetration is usually very small and analysis of either blood or urine can be problematic. Classic experiments (Feldman and Maibach, 1970) utilised radiolabelled materials. However, there can be problems in analysing the data since the analytical technique does not permit differentiation between the parent drug and its metabolites. Other difficulties arise in the choice of the model. Human studies would be ideal but in the case of new drug entities or materials such as pesticides there are toxicological implications. As a result of the difficulties in conducting volunteer studies, many investigations have used animal models. These are useful but it should be stated that the results cannot always be extrapolated to predict in vivo absorption in man. In any animal model there will be species differences in both the physiological and biochemical characteristics. Despite these problems the rabbit remains a useful model and has been used in this study in order to augment in vitro data obtained using conventional procedures.

A series of model compounds has been chosen to span a range of physicochemical properties. Salicylic acid and its methyl, ethyl, phenyl and glycol esters were considered to cover a suitable spread of hydrophilic/lipophilic characteristics. Representative topical bases were chosen and include Plastibases, Carbopol gels and polyethylene glycols (PEGs) complex formulations were avoided in order that the number of potential physicochemical interactions was minimised.

## Materials and Methods

*Materials*

All materials were used as supplied. Plastibase samples were provided, as a gift, by E.R. Squibb Ltd. Carbopol 940 was a gift from B.F. Goodrich Ltd. Glycol salicylate was a gift from Boots plc. PEGs 200, 600, 1000 were supplied by BDH, Poole; salicylic acid, methyl, ethyl and phenyl salicylate, phthalic acid, methanol, acetic acid, toluene, ethyl acetate and perchloric acid were all of analar grade also supplied by BDH, Poole. PEGs 1500 and 2000 were supplied by Hoechst.

*Release studies*

Release of the salicylates from the bases was determined using the method described by Billups and Patel (1979). The membrane used to separate the base from the receptor phase (normal saline) was polydimethylsiloxane (supplied by Lepetit Pharmaceuticals Ltd.) of thickness 0.13 mm and area 2.6 $cm^2$. In all experiments data analysis showed that the membrane was not rate-limiting and drug release was controlled by diffusion within the base. The appearance of the salicylate in the receptor was measured by UV spectrophotometry on the removal of samples at 30-minute intervals.

Drug release from the base was analysed by either Eqns. 1 or 2 depending on whether the salicylate was dissolved or present as a dispersion (Higuchi, 1967). If the salicylate was totally dissolved

$$Q = \frac{2Dc_0t^{1/2}}{\pi^{1/2}} \quad (1)$$

where $Q$ is the amount of salicylate released per unit area at time $t$. $c_0$ is the initial concentration of the salicylate in the base and $D$ its apparent diffusion coefficient.

When the salicylate was not completely dissolved, release was analysed according to Eqn. 2.

$$Q = [2c_0c_st]^{1/2} \quad (2)$$

where $c_s$ is the solubility of the drug in the formulation. The aqueous solubilities (0.9% sodium chloride) of the drugs were determined at the temperature of the experiment (30 °C) and are as follows (Al-Khamis et al., 1986): methyl salicylate, 0.95 mg/ml; phenyl salicylate, 0.18 mg/ml; glycol

salicylate, 10.57 mg/ml. The solubility of salicylic acid in Plastibase was taken as 495 μg/ml (Washitake et al., 1972).

Different particle size fractions of salicylic acid were obtained by grinding the supplied powder in an 18-cm ball mill rotated at 30 rpm for 4 h. The particles were then sieved and the micronised powder separated into 4 size fractions using an Alpine Multiplex classifier. The particles were then dispersed in liquid paraffin (presaturated with salicylic acid) and examined microscopically. The diameters of 50 particles were determined from each of 18 samples giving a median value from 900 particles. These are given in Table 1 together with the value determined after the particles had been incorporated into Plastibase 50 W. The percentage decrease in size is greater for the larger particles suggesting that trituration of the particles in the base was a more significant effect than dissolution in decreasing their diameter.

*In vivo*

Male lop rabbits weighing between 3 and 4 kg were used. They were housed individually in a temperature- and humidity-controlled room where the experiments were conducted. Hair was removed from the back area of the ear by careful shaving 24 h prior to the application of the formulation. A plastic ring of internal diameter 1.5 cm was securely fixed to the ear using cyanoacrylate adhesive. The ring was filled with the base, excess base removed by levelling with a spatula and the ring capped with an aluminium disc. The whole assembly was then covered with adhesive tape and the rabbit restrained. Two ml samples of blood were withdrawn from the marginal ear vein at half-hourly intervals for 7 h using a heparinised cannula. The blood samples were centrifuged and the plasma refrigerated, and later assayed for salicylate. Experiments were conducted in triplicate.

*HPLC assay*

A Perkin Elmer 1220 liquid chromatography system was used with a μ-Bondapak $C_{18}$, 5-μm Waters Associates column. Preparation of the plasma sample prior to injection was based on a modification to the methods used by Peng et al. (1978) and Reepmeyer and Kirchhoefer (1979). 1.0-ml samples of plasma were spiked with an appropriate concentration of an internal standard, phthalic acid and acidified with one drop of 85% perchloric acid. Extraction with toluene–ethyl acetate (1 : 1) was performed twice on the samples and the solvent was evaporated using a stream of nitrogen. The residue was dissolved in 0.5 ml of the mobile phase (60% v/v methanol–water adjusted to pH 2.4 with acetic acid) and 100-μl samples injected onto the column. Using this technique, measured values of standard salicylate solutions could be determined to within 1%.

## Results and Discussion

The effect of particle size on release rate where different size fractions of salicylic acid were dispersed in Plastibase 50W is shown in Fig. 1. Using Eqn. 2 it is possible to calculate the effective diffusion coefficients of the salicylic acid in the bases. These are summarised in Table 2. A linear relationship is found between the effective diffusion coefficient and the specific surface area of the salicylate particles (Fig. 2). This confirms the finding of Konning and Mital (1978) and emphasises the importance of using the smallest particle size possible to increase release rate. The thermodynamic activity of the salicylic acid in the different preparations must be the same since the acid is present above its solubility limit. The differences are thus a result of different dissolution rates which will be controlled by the particle size.

The release rates of salicylic acid and salicylate esters from PEGs and simple carbopol gels were studied. Typical results are shown in Fig. 3. The good correlation between the amount of drug re-

TABLE 1

*Particle sizes of salicylic acid recovered from Plastibase 50W*

| Original size (μm) | Size in Plastibase (μm) | % Change |
|---|---|---|
| 118 | 88 | 25.4 |
| 59 | 46.5 | 21.2 |
| 27.6 | 23 | 16.7 |
| 5.8 | 5.05 | 12.9 |

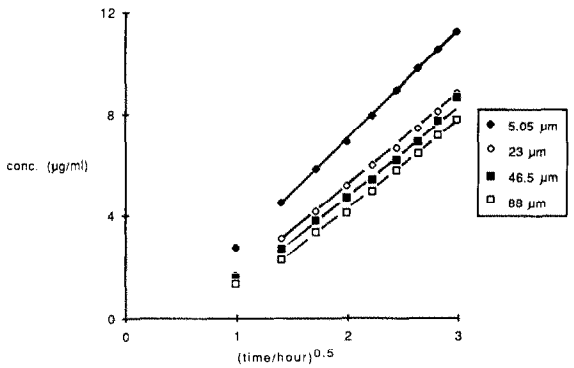


Fig. 1. Relationship between the amount of salicylic acid released from Plastibase 50W and the square root of time for a range of particle size fractions.

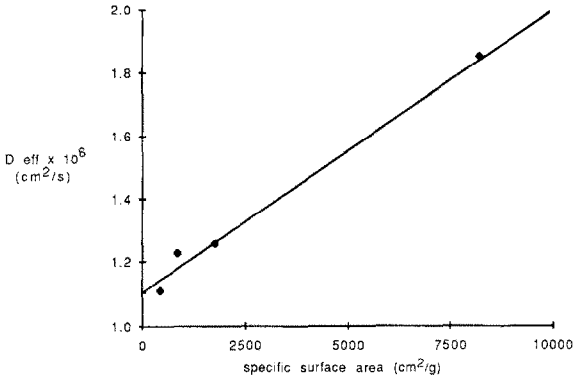


Fig. 2. The effective diffusion coefficient of salicylic acid in Plastibase 50W plotted as a function of the specific surface area of the dispersed particles.

leased and square root of time shows that there is a negligible diffusional resistance from the membrane in the release cells and any associated stagnant diffusion layers. Increasing the molecular weight of the PEG decreases the diffusion of the salicylate. The exact magnitude of this can be seen in Table 3 which gives a compilation of the apparent diffusion coefficients for the systems studied.

Comparing the diffusion coefficients of the individual salicylates within a PEG sample, the methyl and ethyl esters have comparable values. They diffuse more rapidly than the glycol and phenyl esters which, as well as salicylic acid itself, interact with the PEG (Al-Khamis et al., 1982). In the case of glycol salicylate and the free acid the intermolecular forces involved are probably hydrogen bonds.

A detailed description of the results for the carbopol gels has been given recently by Al Khamis et al. (1986). It is apparent that the diffusional barrier in the carbopol systems is very much less than for the PEGs. Indeed the salicylate diffusing in carbopol experiences a microviscosity which is not dissimilar to that of free water.

The different formulations given in Table 3 were applied to the ear of lop rabbits and plasma levels monitored over a 7-h period. The effect of altering the salicylate concentration is given in Fig. 4. There is a characteristic lag time followed by a rapid rise in the plasma concentration which peaks between 5 and 6 h. The type of formulation has an effect on the observed plasma concentrations. 10% methyl salicylate in PEG 1500 is equivalent to 5% methyl salicylate dispersed in 1% carbopol. As expected there is a linear correlation between the applied salicylate concentration and the area under the plasma curve (AUC) (Fig. 5). Since only relative comparisons were required the AUCs were determined by drawing the plasma

TABLE 2

*Effective diffusion coefficients of salicylic acid in Plastibase 50W at 37°C*

| Particle size (µm) | $D_{eff}$ ($cm^2\ s^{-1}$) |
|---|---|
| 88 | $1.11\times10^{-6}$ |
| 46.5 | $1.23\times10^{-6}$ |
| 23 | $1.26\times10^{-6}$ |
| 5.05 | $1.85\times10^{-6}$ |

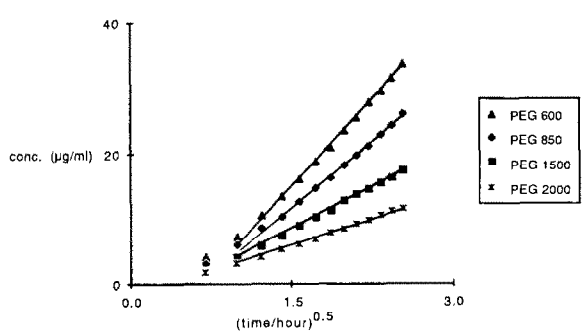


Fig. 3. The release of methyl salicylate (10% w/w) from different PEGs plotted as a function of the square root of time.

TABLE 3

*Apparent diffusion coefficient of salicylate from PEGs and carbopol gels for different concentrations of the drug at 30 °C*

| Ointment base | Drug conc. (% w/w) | Methyl salicylate ($D \times 10^{-7}$ cm$^2$ s$^{-1}$) | Glycol salicylate ($D \times 10^{-9}$ cm$^2$ s$^{-1}$) | Ethyl salicylate ($D \times 10^{-7}$ cm$^2$ s$^{-1}$) | Phenyl salicylate ($D \times 10^{-9}$ cm$^2$ s$^{-1}$) | Salicylic acid ($D \times 10^{-9}$ cm$^2$ s$^{-1}$) |
|---|---|---|---|---|---|---|
| PEG600 | 2 | 2.19 | – | 1.73 | – | – |
| | 5 | 1.89 | – | 1.46 | 1.88 | – |
| | 8 | – | – | 1.58 | 1.32 | – |
| | 10 | 1.80 | 6.20 | 1.55 | 1.16 | 1.96 |
| PEG850 | 2 | 1.66 | 6.90 | 0.51 | – | – |
| | 5 | 1.05 | 7.74 | 0.55 | – | – |
| | 10 | 1.05 | 5.04 | 0.97 | 1.16 | 1.53 |
| PEG1500 | 10 | 0.48 | 3.99 | 0.55 | 0.95 | 1.53 |
| PEG2000 | 10 | 0.20 | 2.84 | 0.12 | 0.60 | 0.97 |
| Carbopol | 2.0 | 96 | – | 55 | – | – |
| 0.5% w/w | 5.0 | 97 | 2650 | 68 | – | – |
| | 8.0 | 132 | – | 94 | 500 | – |
| Carbopol | 1.0 | – | 550 | – | – | – |
| 1.0% w/w | 2.0 | 70 | 1210 | 37 | – | – |
| | 3.0 | – | 1360 | – | – | – |
| | 4.0 | – | 1570 | – | – | – |
| | 5.0 | 80 | 1970 | 38 | 243 | – |
| | 8.0 | 85 | – | 62 | 222 | – |
| | 10.0 | 105 | – | – | 557 | – |
| Carbopol | 2.0 | 58 | – | 9 | – | – |
| 2.0% w/w | 5.0 | 70 | 1720 | 17 | – | – |
| | 8.0 | 77 | – | 25 | 308 | – |

time profile from 0 to 7 h, cutting round the resulting graph and weighing it. The AUC values are thus expressed in arbitrary units.

Fig. 6 shows the plasma time course after the application of methyl salicylate and salicylic acid in a variety of PEGs. The plasma levels achieved are higher from the formulations containing methyl salicylate which is a consequence of both better release of the methyl ester from the base and also more favourable partitioning characteristics into the stratum corneum. For both model drugs there is a linear relationship between the plasma AUC and the nominal molecular weight of the PEG (Fig. 7). The diffusion coefficients of methyl salicylate in the different molecular weight PEGs vary by almost a factor of 10 and this may be

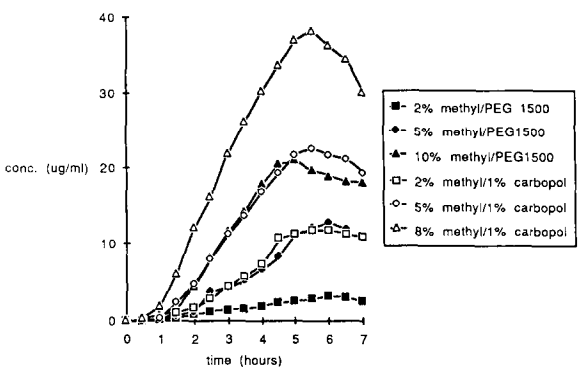


Fig. 4. Plasma salicylate concentration–time profiles for methyl salicylate in different formulations.

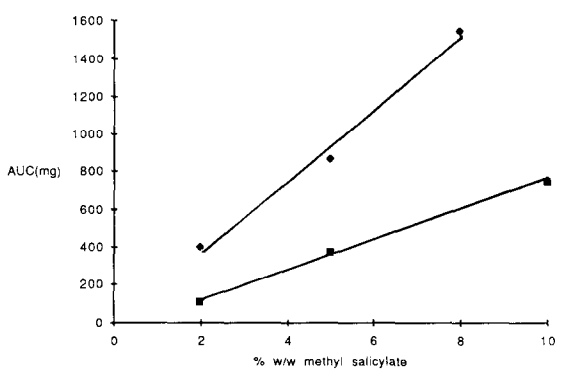


Fig. 5. The relationship between the plasma–time AUC (arbitrary units) and the concentration of applied salicylate.

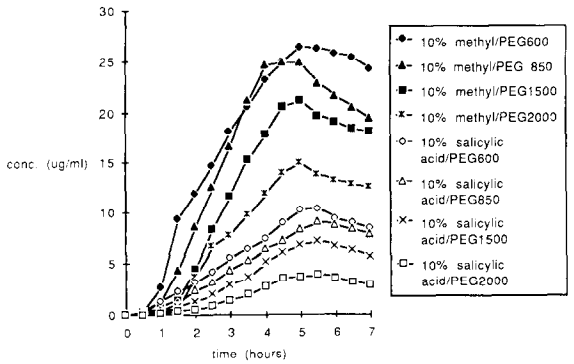


Fig. 6. Plasma salicylate concentration–time profiles for methyl salicylate and salicylic acid in different PEG formulations.

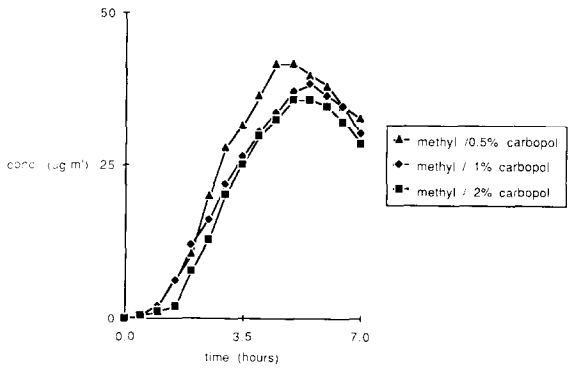


Fig. 8. Plasma salicylate concentration–time profiles for methyl salicylate dispersions in different carbopol formulations.

expected to elicit a larger difference in the plasma levels. However, these will be moderated by partitioning into the skin (any changes in the thermodynamic activity of the drug) and also by the formulation potentially affecting the barrier function of the skin. Separation and quantification of these effects is complex but general trends can be observed.

In the case of the formulations of methyl salicylate dispersed in carbopol gels, the thermodynamic activity of the salicylate will be constant. Altering the carbopol concentration has a small effect on the diffusion coefficient of the salicylate and only a small change is observed in the plasma levels (Fig. 8). These levels do not exhibit a marked difference and formulating with carbopol between 0.5 and 2% has little effect on the observed plasma concentrations. Release from the gels is rapid and small changes in the polymer concentration only slightly affect the microviscosity experienced by a diffusing drug.

Fig. 9 demonstrates that when 10% glycol and phenyl salicylate are applied topically in PEG 1500 similar plasma profiles are obtained. For these two compounds the diffusion coefficients in PEG 1500 are of a similar order of magnitude and much less than the values in carbopol. The same graph illustrates that formulation of these salicylates in a simple carbopol gel produces very much higher plasma levels. This results from both increased diffusion in the gel and increased thermodynamic activity of the substance. These profiles also demonstrate the effect of the formulation on the time to reach maximum plasma levels. For the two carbopol formulations $t_{max}$ occurs at 4.2 h

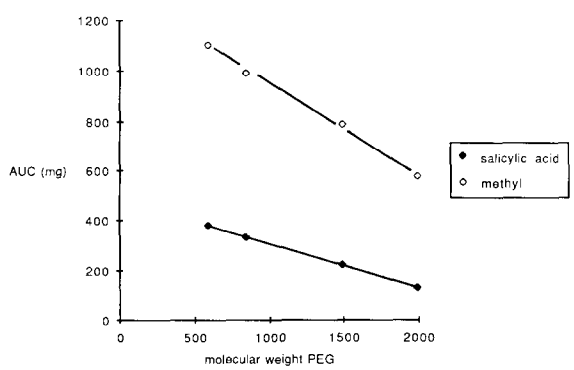


Fig. 7. Relationship between the AUC for methyl salicylate and salicylic acid as a function of the molecular weight of the PEG.

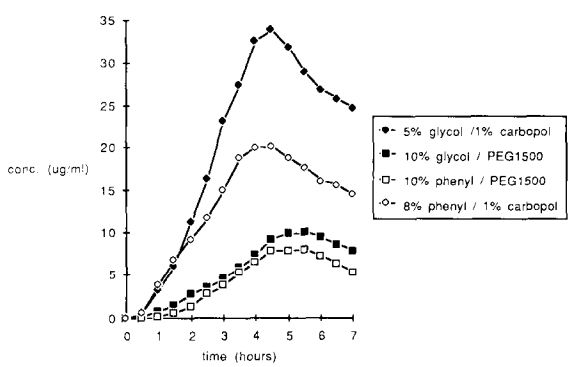


Fig. 9. Plasma salicylate concentration–time profiles for glycol and phenyl salicylate.

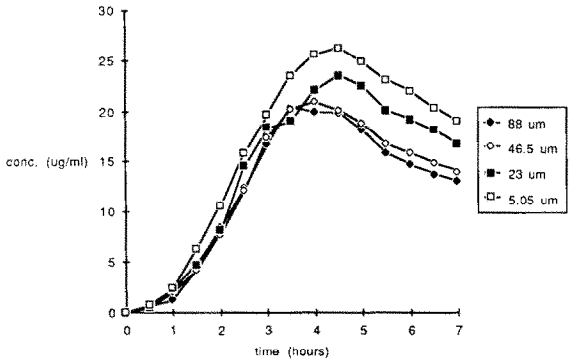


Fig. 10. Plasma salicylate concentration–time profiles for salicylic acid dispersed in Plastibase 50W. The effect of particle size is shown.

which may be contrasted with 5.3 h for the PEG bases. Diffusion of the salicylates within the carbopol gels is much faster than the PEG systems (Table 3) and provides a more rapid attainment of the peak plasma levels.

Particle size effects are shown in Fig. 10: plasma salicylate levels are affected by the particle size to a small extent and the area under the plasma time curve is related to the measured effective diffusion coefficient (Fig. 11).

The data presented above demonstrate the complexities of investigating percutaneous absorption in vivo. A rabbit model was chosen to study formulation effects and this has been achieved. Rabbit skin is more permeable than human skin and thus formulation effects are more evident

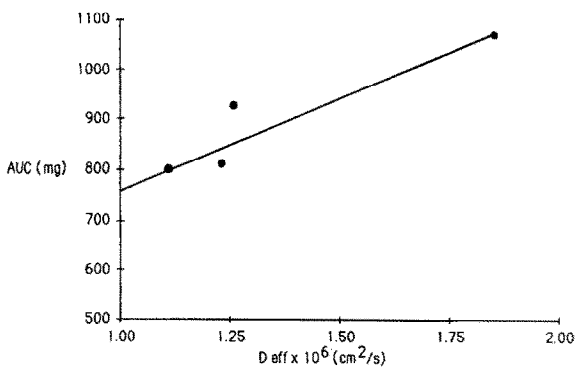


Fig. 11. Relationship between the AUC and the effective diffusion coefficient for the different particle size dispersions of salicylic acid in Plastibase 50W.

(Wester and Maibach, 1985). The high permeability of rabbit skin is seen by considering Fig. 4. Plasma levels rise quite rapidly, the lag time being less than 1 h. Peak concentrations are produced between 5 and 6 h after application followed by a fairly rapid fall. This fast decline indicates that rabbit does not have a large storage capacity for methyl salicylate.

It is often difficult to resolve the operational physicochemical factors which control in vivo plasma levels. The results presented in Fig. 4 illustrate this. The formulations of PEG contain methyl salicylate in solution, increasing the solute concentration increases its chemical potential and thus there is a linear increase in the AUC (Fig. 5). For carbopol, however, methyl salicylate is above its solubility limit and is dispersed. Therefore the thermodynamic activity is constant. The diffusion coefficient of the salicylate at the different concentrations is relatively constant (Table 3) and thus the 'escaping' tendency of the salicylate into the skin for the 3 formulations should be the same. Under these conditions it would be expected that the plasma concentrations should be the same. Fig. 4 demonstrates that they are not and this must reflect the absolute amount of dispersed methyl salicylate that is in direct contact with the skin. In this instance a linear relationship between the applied concentration and the AUC would be expected as is found in Fig. 5.

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