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Effect of PGML Excipient Mixture in a Transdermal System on the in Vitro Transport of Estradiol Across Skin

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Purpose. To measure the effect of a combination of excipients from a silicone based pressure sensitive adhesive (PSA) on drug transport across skin.
Methods. Partitioning of propylene glycol monolaurate (PG-ML) from silicone PSA and a solution formulation into the stratum corneum (SC) was measured using radiolabeled PG-ML. Transport of a model drug, estradiol, as well as PG-ML across skin were measured in vitro using heat separated epidermis from human cadaver skin.
Results. The PG-ML partitioning into SC showed a saturation and was independent of the formulation. The local dielectric of the lipid bilayers of the SC showed an increase as a result of PG-ML uptake, as determined by the decrease in fluorescence lifetime of a lipophilic probe incorporated into the SC. However, there was no alteration of lipid packing in SC.
Conclusions. The PG-ML and estradiol transport showed a good correlation over 3 days, suggesting that the two species are co-transported across the epidermis.

KEY WORDS: transdermal drug delivery; pressure sensitive adhesive; transport enhancement; fluorescence spectroscopy; estradiol.

INTRODUCTION

Transdermal drug delivery has become an attractive alternative in controlled drug delivery (1). The stratum corneum (SC) of the epidermis provides the major barrier to percutaneous penetration of drugs (2,3). This diffusional barrier is often overcome with the aid of excipients in the transdermal formulations (4,5). These excipients are thought to act as solubilizers, fluidizers, plasticizers or vehicles. The excipient could increase the solubility of the drug in the adhesive, thereby increasing the concentration in the delivery system in situations where the drug is present in a powdered form in the adhesive. It could partition into the skin and perturb the highly organized lipid bilayers of the SC, increasing the permeability of the skin (4). The excipient could act as a plasticizer in the adhesive, increasing the diffusion of the drug in the adhesive. It could also act as a solvent or as a vehicle, facilitating the transport of the drug across skin. Excipients are used individually or in combination in transdermal systems to provide any one or a combination of the above effects resulting in an enhanced delivery of drugs across skin (4,6).

One of the first steps in the development of a transdermal drug delivery system is to perform a formulation feasibility and optimization study from a solution formulation before exploring a suitable pressure sensitive adhesive (PSA) platform. Quite often, the transdermal flux of the drug observed from a PSA matrix formulation is less than from a solution formulation. The literature is replete with reports from solution formulations on enhancer effects (4-6). However, there are only few studies reported from solid matrix formulations (7).

In the present investigation, we have measured the uptake of an excipient, propylene glycol monolaurate, into skin and its transport across skin from a silicone based PSA and a liquid formulation. Propylene glycol monolaurate, a fatty acid ester, is used to enhance the transport of drugs from transdermal systems (8,9). The transport of a model drug, estradiol, was measured to determine if there was any correlation between the transport of drug and excipient from a given formulation. The objective of the present study is to understand the effect of a combination of excipients from a silicone based PSA on skin. Building on our earlier work (10), we have used a frequency-domain fluorescence spectroscopic technique to determine the effect of these excipients on the lipid order of the SC.

MATERIALS AND METHODS

A mixture (PGML) of propylene glycol monolaurate (PG-ML, 52.9% by weight), propylene glycol dilaurate (PG-DL, 32.7%), methyl laurate (ML, 9.2%), and propylene glycol (PG, 5.2%) obtained from Gattefosse (France) was used without further purification. The composition of this mixture was determined by gas chromatography using a HP-5890 gas chromatograph with a Restek RTX-35 capillary column and a FID detector. Radiolabeled PG-ML was synthesized using PG and 1-^{14}C lauric acid (American Radiolabeled Chemicals, Inc., St. Louis, Missouri). One mCi (3.66 mg) of ^{14}C lauric acid was added to a mixture of 2 mg of unlabeled lauric acid and 5.36 mg of PG and the resulting reaction mixture of lauric acid and PG (1:2.5, mole ratio) in hexane was mixed with 0.3M Boron Trifluoride (BF_3) ethyl etherate catalyst in diethyl ether and the reaction mixture was maintained at 70°C for 6h under nitrogen. The excess catalyst was removed by drying under a stream of nitrogen and the ^{14}C PG-ML was isolated from the reaction mixture by preparative thin layer chromatography. The reaction mixture was dissolved in chloroform, applied on a 1 mm thick silica gel plate (Alltech Associates), and eluted in hexane:ethyl ether:acetic acid (69:30:1, by volume). Commercial PGML mixture was used as the standard to identify the different bands. The PG-DL eluted the furthest on the plate, followed by lauric acid and PG-ML eluted about one-third the distance from the origin. The ^{14}C PG-ML fraction from this plate was purified on a second plate using a polar solvent system (5% methanol in chloroform). In this solvent system, the ^{14}C PG-ML moved one-half the distance from the origin, while the lauric acid (which was a cross-contamination from the first fraction) was retained close to the origin. The final yield of PG-ML was 0.73 mg or ~ 10%.

Two different donor formulations were used in the up-

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take and transport study. Neat PGML liquid spiked with 4μCi of ^{14}C PG-ML was used as a solution formulation. A silicone PSA formulation was made by adding a known amount of PGML to a silicone solution in Freon (non end capped, 58% solids from Dow Corning) to a final composition of 14% by dry weight. The solution was spiked with 2μCi of ^{14}C PG-ML. The liquid adhesive was stirred for 2h before casting the film at a thickness of 5 mil. A polyester film was used as the backing and as the release liner for the patches. The specific activity of ^{14}C PG-ML in the final formulations were 25.5 μCi/g of PG-ML in the neat PGML liquid and 24.9 μCi/g of PG-ML in the silicone formulation.

Skin uptake and transport studies were performed using Franz cells across a diffusional area of 0.785 cm^2. The estradiol transport studies were done using multiple pieces of skin from different donors, while the rest of the experiments were done using three pieces of skin from the same donor. The patches with the backing material were applied on to pieces of heat separated human epidermis obtained from human cadaver skin, and the receiver fluid was analyzed for ^{14}C PG-ML by liquid scintillation counting, at intervals of 24h for 3 days. The amount of ^{14}C PG-ML left in the patch and in epidermis were also analyzed at each time point. After the flux studies, the epidermis was removed from the diffusion cell, and the adhesive was peeled away. The patches and the backing were extracted in methanol:methylene chloride (1:1, by volume) overnight and analyzed for ^{14}C PG-ML. The epidermis was digested in 1 ml of Solvable (0.5M sodium hydroxide, NEN Research Products) overnight and then counted. In one of the time points, i.e., the third day sample, the SC was separated from viable epidermis by trypsin digestion. The epidermis was placed dermal side down on a filter paper soaked in 1% trypsin for 4 to 6h at room temperature and then at 4°C overnight. The partially digested tissue was agitated in a fresh 0.5% trypsin solution for 1h resulting in a translucent sheet of SC, which was rinsed in distilled water, digested in Solvable and counted for PG-ML. The trypsin digests along with the filter paper were counted for PG-ML in the viable layers. Day 3 samples were analyzed for ^{14}C PG-ML in the SC as representative samples. In the samples where neat PGML was the donor, the epidermis was rinsed three times in 3 ml portions of unlabeled PGML to remove any PG-ML from the epidermis surface before digesting in Solvable. In a separate experiment, SC was separated from pieces of epidermis by trypsin digestion as described above. The SC pieces of known area were then equilibrated with PGML liquid mixture in a scintillation vial. The PGML mixtures was spiked with ^{14}C PG-ML to a specific activity of 15 μCi/g of PG-ML. After 72h equilibration, the pieces of SC were rinsed three times in unlabeled PGML to remove any ^{14}C PG-ML from the surface, and the tissue was treated with trypsin identical to the method described above. After rinsing in distilled water, the SC pieces were digested in Solvable and analyzed for ^{14}C PG-ML. The trypsin fractions along with the filter paper and the water rinses were pooled and counted for ^{14}C PG-ML as a check for the amount of PG-ML that could have leached out during the different experimental steps. In the estradiol transport experiments, the drug was present at 5% by weight in the silicone PSA formulation and was present as a saturated solution in the PGML liquid formulation. The estradiol in the receiver fluid was analyzed by reverse-phase HPLC using a C-18 column.

For fluorescence experiments, the epidermis was treated similar to the transport studies, but the donor formulation did not contain any radiolabeled PG-ML or estradiol. The epidermis was equilibrated for 18h in a side-by-side diffusion cell, with a donor solution of a fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) in water. The aqueous solution of DPH was prepared by adding 5μl of 2mM DPH solution in ethanol to 1.5 ml of water corresponding to a final concentration of 6.7μM. After pretreatment with DPH, the epidermis was treated with the PGML liquid and the silicone PSA formulations separately, as described above in the uptake studies. At the end of the treatment period (24h to 72h), the epidermis was removed from the diffusion cell, mounted on an aluminum block and placed in the sample holder for spectral acquisition. The samples in which the epidermis was treated with the silicone-based formulations, the spectral acquisition was performed with the adhesive on the epidermis. All fluorescence measurements were performed using an SLM-Aminco MHF 4850 spectrofluorimeter. Fluorescence lifetimes and anisotropy were measured using the phase modulated 325 nm line of a HeCd laser as described elsewhere (10 - 12).

RESULTS

Skin Uptake Data. The uptake of PG-ML into epidermis showed tissue saturation after day 2 from the silicone but not from the PGML donor, as seen in Fig 1. The distribution of PG-ML between the SC and the viable layers of epidermis is shown in Table I. The amount of PG-ML per cm^2 of SC was comparable from both the silicone and the PGML formulations, suggesting tissue saturation. The amount of PG-ML per unit area of the viable layers of the epidermis was three-fold higher from the PGML formulation compared to the silicone. The amount of ^{14}C PG-ML lost from the SC during trypsin treatment and water rinses was 11.5 ± 2.1% of the total ^{14}C PG-ML in the SC, which is within the standard deviation of the SC-uptake data shown in Table I. The data in Table I are not corrected for this PG-ML loss.

Transport Data. The transport data for PG-ML across epidermis is shown in Fig 2. The amount of PG-ML transported across epidermis gradually increased with time up to 3 days from both silicone and PGML liquid formulations. The amount of PG-ML transported across epidermis from the PGML mixture was six-fold higher than that from the silicone PSA formulation. Estradiol transport across epidermis from these two formulations is shown in Fig 3. The estradiol transport from the PGML liquid formulation was about 15 times that from the silicone PSA formulation. The drug transport across the epidermis from these two formulations showed a good correlation with the PG-ML transport over a period of 3 days, as seen in Fig 4.

Fluorescence Data. Table II shows changes in lifetime and limiting anisotropy for DPH in epidermis treated with different formulations. The lifetime decreased after 3 days of exposure to PGML from both formulations. The decrease in lifetime was observed even after 24h exposure to PGML from the neat liquid and was twice that from the PSA-treated epidermis. The limiting anisotropy showed no decrease after

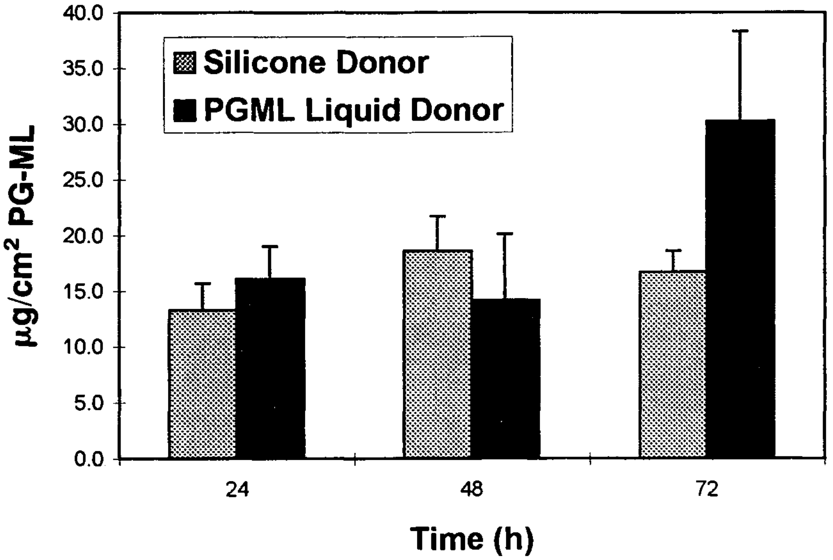

Fig. 1. Amount of propylene glycol monolaurate partitioning into the epidermis from silicone PSA and PGML liquid formulation (n = 3).

treatment with the PGML liquid formulation. The limiting anisotropy measurements in samples treated with the silicone formulations did not yield any useful information due to interference from the overlying silicone adhesive. Spectral data from an earlier study (10) using oleic acid-containing formulation showed large drops in lifetime and limiting anisotropy and are included in Table II for comparison.

DISCUSSION

We concentrated on following the fate of the major component, viz. PG-ML, as representative of the behavior of the PGML mixture. Among the different components, PG is polar while the other components are lipophilic. However, PG constitutes only 9% of the mixture, which translates into 1.25% in the silicone formulation containing 14% of PGML. Comparatively, PG-ML constitutes 53% of the PGML mixture and represents 7.4% of the PSA system. PG is known to act as a cosolvent, showing a synergy with other penetration enhancers in terms of flux enhancement (4,8,13). This synergy is observed only at very high PG concentrations, when the PG is used as the vehicle or solvent (4,8). Therefore, PG transport may not be relevant to drug transport at low concentrations of PG as in the present formulations. Thus, PG-ML is a useful representative molecule to monitor the fate of mixture of lipophilic excipients in the PSA matrix. PG-ML is freely miscible in PGML mixture, while the 14% PGML-containing silicone did not show any signs of phase separation when examined by light microscopy. Thus, it is reasonable to assume that the PG-ML was present below unit activity in both formulations. However, there was much higher amount of PG-ML in the PGML liquid formulation, than in the silicone. The PG-ML partitioning into epidermis at 72h was higher from neat PGML mixture than from the PSA, which could be explained in terms of the larger driving force resulting from a higher concentration gradient and therefore higher thermodynamic activity of PG-ML in neat PGML as compared to the silicone formulation.

The uptake of PG-ML by SC, the barrier layer of epidermis, is more important in terms of flux enhancement than the uptake by epidermis. The saturation of SC suggests that the effect of PG-ML on lipid bilayers of the SC should also be saturated. This was further confirmed by the biophysical investigation of the effect of these two formulations on the

Table I. Distribution of PG-ML in the epidermis

Formulation	Stratum corneum (µg/cm^2)	Viable epidermis (µg/cm^2)	Ratio[a]	Stratum corneum (% Total)
Silicone	9.12 ± 1.02	7.58 ± 2.35	12.03	54.6 ± 10.0
PGML	7.81 ± 2.52	22.35 ± 5.55	3.49	25.9 ± 1.9

[a] The ratio of PG-ML in SC to viable epidermis was calculated after normalizing for unit thickness of the different layers.

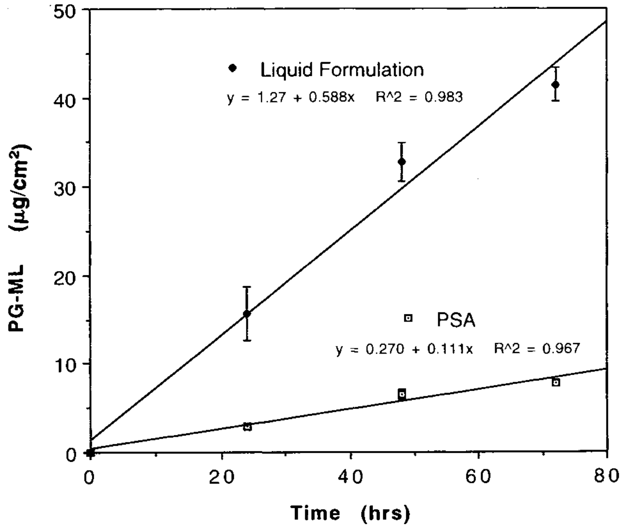

Fig. 2. Transport of propylene glycol monolaurate across human epidermis from silicone PSA (□) and PGML liquid (◆) formulations (n = 3).

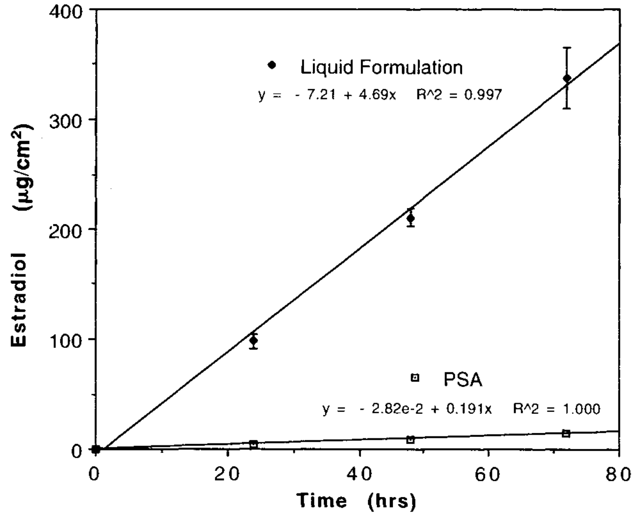

Fig. 3. Transport of estradiol across human epidermis from silicone PSA (▫) and PGML liquid (◆) formulations (n = 12).

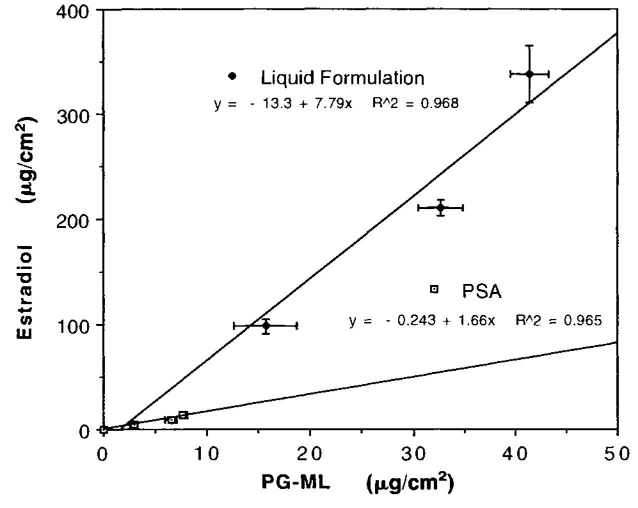

Fig. 4. Correlation of transport of propylene glycol monolaurate and estradiol over 72h, from silicone PSA (▫) and PGML liquid (◆) formulations.

Table II. Fluorescence Parameters of DPH in Epidermis Treated with Different Formulations

Formulation	Equilibration Time (h)	$\Delta\tau$ ± 0.2 nsec	r_∞ ± 0.005
Water	24	0	0
Placebo silicone patch	72	−0.5	—
Silicone (14% PGML)	72	−1.1	—
Liquid PGML	24	−2.3	0
5% OA in 40% ethanol	24	−2.2	−0.1[a]

[a] From ref (10); $\Delta\tau$, r_∞ – changes in fluorescence lifetime and limiting anisotropy compared to the control formulation (water).

SC lipids. Changes in fluorescence lifetime of DPH is a measure of changes in the local dielectric of the medium (10). Thus the decrease in fluorescence lifetime, shown in Table II, indicated that the local dielectric in SC bilayers increased upon treatment with PGML and this effect was observed in both formulations. The larger drop of about 2.3 nsec in lifetime in the epidermis treated with liquid PGML formulation, as compared to about 1.1 nsec drop in the epidermis treated with silicone formulation suggests that the dielectric of lipid bilayers increased more with the application of solution formulation. This could be due to increased transport of PG-ML across SC as seen in Fig 2. This is purely speculative at this point as we have not investigated large number formulations to generalize this observation. The reason for the decrease in the lifetime in epidermis treated with the placebo patch is not clear, although the occluded condition from such treatment could increase hydration and therefore the dielectric of the lipid bilayers. Nevertheless, the perturbation resulting from the partitioning of PG-ML into SC is clearly evident from both the solution and the silicone formulations.

From a thermodynamic consideration, if the SC uptake of PG-ML reached saturation, then the two phases, i.e. the SC and the donor phase must be at the same, unit activity at equilibrium. But PG-ML is freely miscible in the PGML mixture and makes up only 52.9% of the mixture and therefore is not at unit activity. Thus the saturation of PG-ML uptake that is observed in this study is due to a volume constraint of the intercellular lipid matrix of the SC and is not a thermodynamic equilibrium. In other words, the PG-ML does not reach its solubility limit in the lipid bilayers. This is further supported by the calculated value of the Hildebrand solubility parameter (δ) for PG-ML, which is around 9.06 H which compares well with the estimated δ of 9.7 H for SC (14). Thus, the lipophilic PG-ML would be expected to be freely miscible with the nonpolar lipids of the SC. However, the lipid bilayers accommodate only a small number of exogenous molecules such as PG-ML in their packed configuration in the tissue, as measured in this study.

The distribution ratio of PG-ML per unit area per unit thickness of the different layers is shown in Table I. The SC consists of 10 to 15% by weight of lipids (15), while the viable layers are predominantly aqueous. The SC is about 15 µm thick while the viable epidermis is about 150 µm thick. Thus it is not surprising that the SC is able to accommodate more PG-ML (which is lipophilic) per unit volume compared to the viable epidermis. While the amount of PG-ML partitioning into SC is independent of the formulation, the relative ratio is dependent on the formulation.

The partitioning of PG-ML did not show any effect on the anisotropy values. The limiting anisotropy is a measure of the lipid packing order in the SC and other bilayer membranes (12,16). Thus the anisotropy data in Table II suggest that the PG-ML does not perturb the epidermis lipid packing to the same extent as the oleic acid. It is interesting to compare the mole ratio of PG-ML to lipid in SC to that of oleic acid. Assuming the mass of SC to be 1mg/cm^2 (based on the average weight determined for pieces of porcine SC in our laboratory), assuming an average molecular weight of 600 for the SC lipids (17), and based on the average amount of PG-ML partitioning into SC (Table I), we can calculate a mole ratio of 1:51 (PG-ML:SC lipid). The amount of oleic acid partitioning into SC was shown to be 3:100 (oleic acid:SC lipid) in an earlier study (10). Although the amount of PG-ML uptake is significant and is about 67% of the oleic acid uptake, the perturbation in terms of lipid packing in the SC bilayers is almost completely absent for the PG-ML. However, there is a significant increase in estradiol flux from PGML-containing PSA formulation compared to the PSA formulation without PGML, as shown in Fig 5. Also, there was a strong correlation between estradiol transport and PG-ML transport, as shown in Fig 4. The drug loading is a key factor in this comparison. Estradiol was present above its solubility in silicone. It was present as a saturated solution in PGML liquid formulation. Thus, the drug was at unit activity in both formulations. However, more PG-ML and therefore more drug was transported across epidermis from the liquid formulation. This is because of the higher driving force from the solution formulation for PG-ML. Perturbation of lipid packing in the barrier layer by PG-ML does not seem to play a major role in flux enhancement. The increased PG-ML transport could facilitate increased transport of estradiol by co-transporting with it or by increasing the solubility of the drug in skin.

In conclusion, PG-ML from a mixture of mono-, di-, and methyl-laurate and PG in PGML seem to perturb the SC lipids minimally. A strong correlation seen between the transport of PG-ML and estradiol suggests that the two species are co-transported across the epidermis. There is a tissue saturation effect in the uptake of PG-ML that is independent of the formulation. The increased transport of estradiol and PG-ML from a solution formulation could be explained in terms of a higher concentration gradient compared to a silicone formulation. The challenge to the formulation scientist trying to achieve higher drug flux by the use of excipients is the limit imposed by the ability of the PSA matrix to accommodate a given amount of excipient, without compromising adhesion and other bulk properties of the PSA.

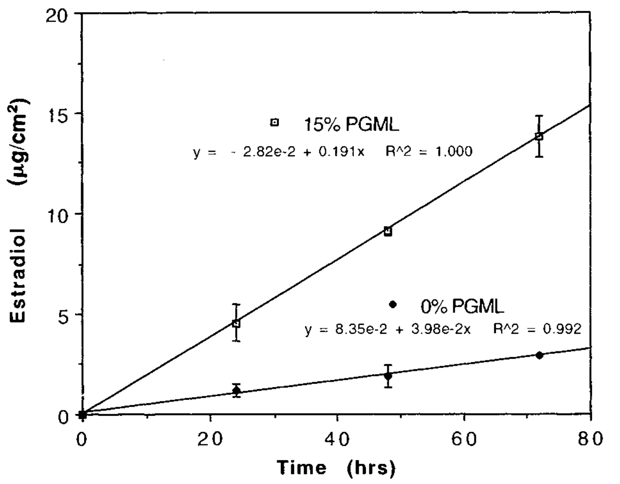

Fig. 5. Transport of estradiol across human epidermis from silicone PSA formulation containing 0% (◆) and 15% (□) PGML by weight (n = 12).

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