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Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions

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Abstract

In vitro assays were performed to investigate the effectiveness of transdermal administration of methotrexate (MTX) by iontophoretic delivery from two types of hydrogel and passive delivery from two types of microemulsion. Both iontophoretic delivery of MTX from hydrogels and passive delivery from microemulsions were more effective than passive delivery from aqueous solutions of the drug. In the iontophoretic delivery assays, the type of hydrogel used and the concentration of the drug in the loading solution had little influence on effectiveness of delivery. In the passive delivery assays, we used both water/oil (w/o) and oil/water (o/w) microemulsions: effectiveness of delivery was higher from o/w systems. At the end of all assays, significant amounts of MTX were detected in the skin. These results suggest that both hydrogels and microemulsions may be of value for the topical administration of MTX in the treatment of psoriasis.

Keywords: Methotrexate; Iontophoresis; Hydrogel; Microemulsion; Psoriasis

1. Introduction

For certain drugs, transdermal delivery offers a number of advantages with respect to oral or parenteral administration. However, only a small minority of drug molecules are able to passively penetrate the skin (Thacharodi and Rao, 1994). Ionic, neutral and/or polar molecules typically show limited skin penetration ability (Rao et al., 1993; Kim et al., 1993).

Methotrexate (MTX) is a folic acid antagonist with antineoplastic activity. It is also effective for the treatment of psoriasis when administered by the oral or parenteral route over long periods of time. However, the systemic use of this drug may provoke any of the numerous side effects, notably hepatotoxic effects (Bookbinder et al., 1984; Van Dooren-Greebe et al., 1994). To reduce such effects, it would clearly be preferable to administer MTX topically (Hwang et al., 1995). A major

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problem is that the drug is hydrosoluble and has a high molecular weight (454.56 g/mol), and is mostly in dissociated form at physiological pH: its capacity for passive diffusion is thus limited. However, techniques such as iontophoresis (Singh and Singh, 1995), or the use of appropriate vehicles such as hydrogels or microemulsions (Encyclopedia of Pharmaceutical Technology, 1994; Lu and Jun, 1998) may enhance transdermal delivery of this drug.

The aim of the present study was to evaluate hydrogels and microemulsions as vehicles for the topical administration of MTX. This forms part of our long-term efforts to develop a topical dosage form suitable for the local treatment of psoriasis.

In iontophoretic delivery, an electric field is applied across the skin, enhancing the penetration of ionized, neutral or polar molecules (Rao et al., 1993; Kim et al., 1993). Previous studies have demonstrated that iontophoretic delivery is effective for enhancing the transdermal penetration of MTX (Álvarez-Figueroa et al., 2000). The development of more convenient reservoir systems is clearly desirable, and one notable area of interest has been the use of drug-containing hydrogels. Formulation of the drug as a hydrogel, rather than a solution, facilitates drug handling and, in the case of iontophoretic delivery, allows the patient to remain ambulant.

Hydrogels have attracted increasing attention in recent years, in view of their swelling behaviour, adhesiveness and biocompatability. Incorporation of drugs into hydrogels permits modulation of their release kinetics (Kim et al., 1992; Vakkalanka et al., 1996; Falamarzian and Varhosaz, 1998).

Microemulsions are dispersions of two immiscible components stabilized by a third, amphoteric, component. They are recognized as appropriate vehicles for the administration of drugs (Osborne et al., 1991; Encyclopedia of Pharmaceutical Technology, 1994; Thacharodi and Rao, 1994). The use of a microemulsion as vehicle may enhance transdermal penetration by various mechanisms. Many molecules are solubilized in microemulsions. In addition, microemulsions induce a change in the thermodynamic activity of the drugs they contain, modifying their partition coefficient, and thus favour penetration of the stratum corneum. Furthermore, their component surfactants reduce the functional barrier of the stratum corneum. This latter function may be more or less important, depending on the nature of the surfactant used (Delgado-Charro et al., 1997).

2. Materials and methods

2.1. Reagents

Methotrexate hydrate (98%), Ag wire (99.99% pure, 1.0 mm diameter), AgCl (99%), Pt wire (99.99% pure, 0.5 mm diameter), phosphoric acid (98%), ethyl oleate (70%), Tween 80, Span 80, 1-2 octanediol (98%), myristic isopropyl ester (98%) and HEPES (99.5%) were all from Sigma-Aldrich Química S.A. (Spain). Acrylamide ($>99\%$), methanol (HPLC quality) and acetonitrile (HPLC quality) were from Merck S.A. Acrylic acid ($>99\%$) and *N*,*N*′-methylenebisacrylamide ($>98\%$) were from Fluka. 2-2′-Azobis (2-aminopropane) dihydrochloride (V50) was from Wako Chemicals, USA. Labrasol® (polyethylene glycol-8 caprylate/caprate) and Plurol Isostearique® (polyglyceryl isostearate) were from Gattefossé (Lyon, France). All the other reagents (NaCl, ethanol, HCl, NaOH, Na_3PO_4) were of analytical grade.

2.2. MTX hydrogels

Hydrogels were prepared with the monomers acrylic acid and acrylamide. In preliminary trials we used different monomer ratios and different amounts of cross-linker, and finally selected two types of hydrogel, one prepared with acrylic acid only, and the other with 1:1 acrylic acid:acrylamide. The selection criteria were viscosity, ease of synthesis, and reproducibility of synthesis (Katime et al., 1999, 2000). The two types of hydrogel were prepared with the same amount of cross-linking agent.

The acrylic acid-only hydrogel was prepared by dissolving 0.02 g of *N*,*N*′-methylenebisacrylamide (the crosslinker) in 8 ml of distilled water in a test

tube, then adding 3 g of acrylic acid, then bubbling with N_2 for 10 min. In another tube, 0.03 g of V50 was dissolved in 2 ml of distilled water, and the system was then bubbled with N_2 for 2 min. The V50 solution (i.e. the initiator) was then added to the monomer solution, and the tube was capped with a septum seal and bubbled with N_2 for 2 min. The hydrogels were then washed for 1 week, with constant changing of the water. They were then cut, dried and polished to obtain hydrogels with a smooth and homogeneous surface (Álvarez-Figueroa et al., 1999).

The 1:1 acrylic acid:acrylamide hydrogel was prepared by the same procedure, with the acrylamide (1.5 g) added to the crosslinker solution first, then the acrylic acid (1.5 g) (Álvarez-Figueroa et al., 1999).

For loading with MTX, the hydrogels were placed in contact for 1 week with 30 ml of buffer solution (25 mM HEPES + 50 mM NaCl) containing 120 or 200 μg/ml of MTX.

2.3. Microemulsions

The microemulsion systems studied in this work have been previously characterized in our laboratory (Liz-Marzán, 1992; Delgado-Charro et al., 1997; Baroli et al., 2000). An important characteristic of these systems is that they employ non-irritant surfactants and co-surfactants, suitable for topical application.

The two systems used were:

1. Surfactant/cosurfactant 3:1 v/v Labrasol®:(Plurol Isostearique®); oil phase ethyl oleate; aqueous phase 154 mM NaCl pH 7.4.
2. Surfactant/cosurfactant 3:1:1.2 v/v/v (Tween 80):(Span 80):(1,2-octanediol); oil phase myristic isopropyl ester; aqueous phase water.

Various microemulsions were prepared, all within the stability zones of the corresponding phase diagrams, as shown in Table 1. The required volume of each component was measured in a measuring cylinder, the components were mixed by shaking, and the mixture was left in a water bath at 37°C until stabilization (about 24 h).

For passive diffusion assays (Section 2.5), the microemulsion was prepared with MTX at 50 or 80% saturation.

2.4. In vitro assays of iontophoretic delivery from MTX hydrogels

Skin was obtained from various regions (neck, hind leg, fore leg, back) of a 2–3-day-old pig that had been killed by cervical dislocation. The skin was frozen at −20°C until use, and thawed about 12 h before each experiment. Muscle and subcutaneous tissues were removed and any hair cut short. The skin was then cut into appropriately sized pieces which were placed in the diffusion cells with the epidermis facing the donor solution. In each series of assays we used skin from all four regions, to minimize inter-assay variability due to variability among skin types.

For these experiments we used vertical passive diffusion cells (area 4.15 cm^2) (Laboratory Glass Apparatus Inc., USA), protected from light and magnetically stirred throughout the experiment. A constant direct current (0.5 mA/cm^2) generated with a Kepco TES 2360LCR apparatus (Kepco Power Supply, USA) was applied. The voltage of the complete circuit and of each cell was measured hourly with a voltmeter (Freak, MY-63). The silver wire used at the anode was introduced

Table 1
Composition of the different microemulsions tested

System	Microemulsion	% oil phase	% aqueous phase	% S/C
I	OE1	10	10	80
I	OE2	10	50	40
I	OE3	20	25	55
II	EM1	10	15	75
II	EM2	25	15	60

into one of the outlets of the receptor compartment. The cathode was a silver disc coated with AgCl (Green et al., 1991), with the same dimensions as the donor hydrogel. The receptor solution was 4 ml of 25 mM HEPES buffer (pH 7.4) + 154 mM NaCl. The experiments were performed at room temperature.

In all experiments, the concentration of MTX in the receptor solution was determined after 10 h, and the amount of MTX in the skin was determined subsequently.

Six replicates of each experiment were performed.

2.5. In vitro assays of passive diffusion from MTX microemulsions

For these experiments we used vertical passive diffusion cells (area 4.15 cm^2), thermostatted at 37°C in a water bath (Selecta, Digiterm, Spain) in the dark. The donor solution was 3 ml of the microemulsion containing MTX. The receptor solution was 4 ml of 25 mM HEPES buffer pH 7.4 + 154 mM NaCl. The receptor solutions were magnetically stirred (HP Variomag Multipoint Electronicrührer) throughout the experiment. The concentration of MTX in the receptor solution was determined after 6 and 24 h of passive diffusion, and the amount of MTX in the skin was determined subsequently.

Six replicates of each experiment were performed.

2.6. Quantification of MTX

For quantification of MTX in receptor solutions, the solution was first passed through a 0.45 μm polyvinyl difluoride filter (Millipore), then evaporated to dryness in a vacuum oven (Heraeus) at 50°C. The dry residue was then dissolved in 1.0 ml of HPLC mobile phase (see below), and the solution was then centrifuged (Sigma 2-15, Laborzentrifugen, Germany) for 10 min at 11 000 rpm. The supernatant was filtered, and MTX was quantified in the filtrate by HPLC, as below.

For quantification of MTX in skin, the skin was first washed with ethanol (2 × 5 ml), then left in 10 ml of ethanol in the dark for 48 h. The ethanol was then filtered through filter paper, and evaporated to dryness at 50°C under vacuum. The dried residue was then dissolved in 1.0 ml of HPLC mobile phase (see below) and centrifuged for 10 min at 11 000 rpm. The supernatant was filtered through 0.45 μm polyvinyl difluoride filters, and MTX was quantified in the filtrate by HPLC, as below.

HPLC quantification of MTX was as per Cercós-Fortea et al. (1997), using a Merck Hitachi high-performance liquid chromatograph with an L-6200[a] pump, an L-4500 diode detector, an AS-4000[a] injector, and a D-6000 interface. The mobile phase was 84:16 (0.05 M phosphoric acid, pH 2.7):acetonitrile, degassed by vacuum filtration. Both the column and the pre-column were Lichrospher 100 RP-18 columns (5 μm pore size) (Merck). Flow rate was 1 ml/min. System temperature was maintained constant at 35°C, and MTX was quantified on the basis of absorption at 303 nm. Sample volume was 50–100 μl. The detection limit was 2 μg/ml.

2.7. Determination of MTX solubility in microemulsions

Excess MTX was added to 10 ml of the microemulsion, and left at 37°C in the dark with magnetic stirring for 163 h. At 24, 48, 72, 96 and 163 h, we took an aliquot of the microemulsion and centrifuged it for 10 min at 11 000 rpm. The supernatant was filtered through 0.45 μm polyvinyl difluoride filters (to remove drug in suspension), and was then diluted with an appropriate volume of ethanol. MTX was quantified in the filtrate by spectrophotometry (Shimadzu, UV-240) at 304 nm. For each microemulsion, six replicate assays were performed.

2.8. Data analysis

Results are cited in the text as mean ± SD. Data were analyzed by analysis of variance, and Dunn's tests or Student–Newman-Keuls tests for subsequent pairwise comparisons. Statistical significance was fixed at $P < 0.05$.

Table 2
Summarized results of the assays of iontophoretic delivery of MTX from hydrogels: amounts of MTX in the receptor solution after 10 h (μg cm^{-2}) (ambient temperature, 0.5 mA cm^{-2}). Values shown are mean ± SD for six assays

Hydrogel	MTX in the receptor solution (loading solution 120 μg/ml)	MTX in the receptor solution (loading solution 200 μg/ml)
Acrylic acid only	1.52 ± 0.41	1.69 ± 0.42
1:1 (Acrylic acid): Acrylamide	0.98 ± 0.32	1.39 ± 0.29

3. Results and discussion

3.1. Iontophoretic delivery from hydrogels

Previous studies have shown that synthetic hydrogels have suitable viscosity for use in topical formulations. In addition, studies of swelling, uptake and MTX distribution have demonstrated that these systems are suitable for the transdermal administration of MTX. Swelling equilibrium is reached after about 80 h, with the amount of water incorporated into the polymeric network being pH-dependent over the range tested (pH 6.0–8.0). The MTX solution penetrates the hydrogel structure homogeneously, and the pH reached inside the hydrogel is appropriate for maintenance of MTX stability (Álvarez-Figueroa et al., 1999).

The results of the assays of iontophoretic delivery of MTX from hydrogels are summarized in Table 2.

These results indicate that hydrogel composition had little effect on the amount of drug crossing the skin. Significant differences were detected between the two types of hydrogel only when the donor solution contained 120 μg/ml MTX. MTX concentration in the donor solution likewise had little effect: a statistically significant difference between the two MTX concentrations was only observed for the acrylic acid:acrylamide hydrogel. Note that we were unable to investigate the effects of loading-solution MTX concentration outside the range tested, because concentrations below 120 μg/ml are not detectable by the HPLC method used, while at concentrations above 200 μg/ml the drug does not distribute homogeneously in the hydrogel, but precipitates at its surface.

In these experiments we used the maximum current advisable for iontophoretic delivery (0.5 mA/cm^2), but nevertheless detected only small amounts of MTX in the receptor solution. Weaker currents would thus be expected to be less effective, and indeed the concentration of MTX in the receptor solution might remain below the detection limit of the HPLC method used.

Previous studies (Weintein et al., 1989; Álvarez-Figueroa et al., 2000) have shown that iontophoresis considerably increases transdermal delivery of MTX from hydrogels, by comparison with passive delivery. Weintein et al. (1989) found that passive diffusion of MTX across human skin, from a solution containing 2% MTX, was about 5 $\mu g/cm^2$ after 48 h.

The apparent transport number (Yoshida and Roberts, 1992) was practically the same for all four assays, ranging from 1.2×10^{-5} to 2.0×10^{-5}. These values are an order of magnitude lower than those estimated for iontophoretic delivery of MTX from solutions (Álvarez-Figueroa et al., 2000), suggesting that the iontophoretic transport of MTX is limited by the presence of highly mobile sodium ions at the anode.

It would also have been interesting to investigate in greater detail the kinetics of iontophoretic delivery of MTX from hydrogels, but (a) MTX concentration in the receptor solution before 10 h was below the HPLC detection limit, and (b) it was not possible to include more silver in the system for sampling beyond this time.

The amounts of MTX retained in the skin in these assays are summarized in Table 3.

The amount of MTX retained in the skin was not significantly affected by either hydrogel composition or MTX concentration in the loading solution.

Table 3
Summarized results of the assays of iontophoretic delivery of MTX from hydrogels: amounts of MTX remaining in the skin after 10 h (μg cm^{-2}) (ambient temperature, 0.5 mA cm^{-2})[a]

Hydrogel	MTX in the skin (loading solution 120 μg/ml)	MTX in the skin (loading solution 200 μg/ml)
Acrylic acid only	1.56 ± 1.13	1.30 ± 0.61
1:1 (Acrilic acid): Acrylamide	2.59 ± 0.64	1.52 ± 0.8

[a] Values shown are mean $\pm$ SD for six assays.

3.2. Microemulsions

On mixing of the different components, translucent and stable microemulsions were formed almost immediately. On addition of MTX, no opalescence was noted, indicating that these systems retained their stability when the drug was added.

Solubilities of MTX in each microemulsion are listed in Table 4. The solubility of MTX was higher in system I (ethyl oleate as oil phase) than in system II (myristic isopropyl ester as oil phase). This may be attributable to the fact that system I is o/w (oil/water), while system II is w/o (water/oil). MTX is hydrosoluble, and thus has higher affinity for the aqueous phase: since this is the external phase in the system-I microemulsions, a greater amount of drug is solubilized.

The results of the assays of passive delivery of MTX from microemulsions are summarized in Fig. 1. The microemulsions contained MTX at 50 or 80% saturation. With all microemulsions studied, and regardless of MTX concentration, at the end of the experiment MTX concentration in the microemulsion remained much higher than in the skin or in the receptor solution, confirming that the quantity of drug present in the microemulsion remained sufficient throughout the assay period.

Considering the results obtained with MTX at 80% saturation, no significant variation in degree of penetration across the skin was detected either between the two systems or within each system among formulations. Considering the results obtained with MTX at 50% saturation, however, statistically significant variation was observed both between and within systems: indeed, the only non-significant differences were between OE2 and EM1, and between EM1 and EM2. These results suggest that formulation factors have marked effects on system efficacy at relatively low MTX concentrations, but not at higher MTX concentrations. Here it is important to note that, for a given MTX saturation (50 or 80%), MTX concentration varied widely (see Table 4). One possible explanation for the more marked effect of formulation factors at 50% saturation is that the magnitude of the effect of MTX concentration on degree of penetration declines with increasing MTX concentration: this might explain, for example, why degree of penetration differed markedly between EM2-50% and OE3-50% (MTX concentration 298 vs 679 μg/ml) but not between EM2-80% and OE3-80% (MTX concentration 476 vs 1086 μg/ml). However, detailed investigation of the effects of formulation factors on degree of penetration is difficult for systems of this type, since it is not possible to vary one factor over a

Table 4
Solubilities of MTX (μg/ml) at 37°C in the different microemulsions, and MTX concentration used (μg/ml) in the skin penetration assays[a]

Microemulsion	MTX solubility	80% saturation MTX concentration	50% saturation MTX concentration
OE1	1275.81 ± 249.26	1021	638
OE2	977.50 ± 79.87	782	489
OE3	1357.87 ± 76.39	1086	679
EM1	669.60 ± 168.72	536	335
EM2	595.23 ± 57.203	476	298

[a] Solubilities values are mean+SD for six assays.

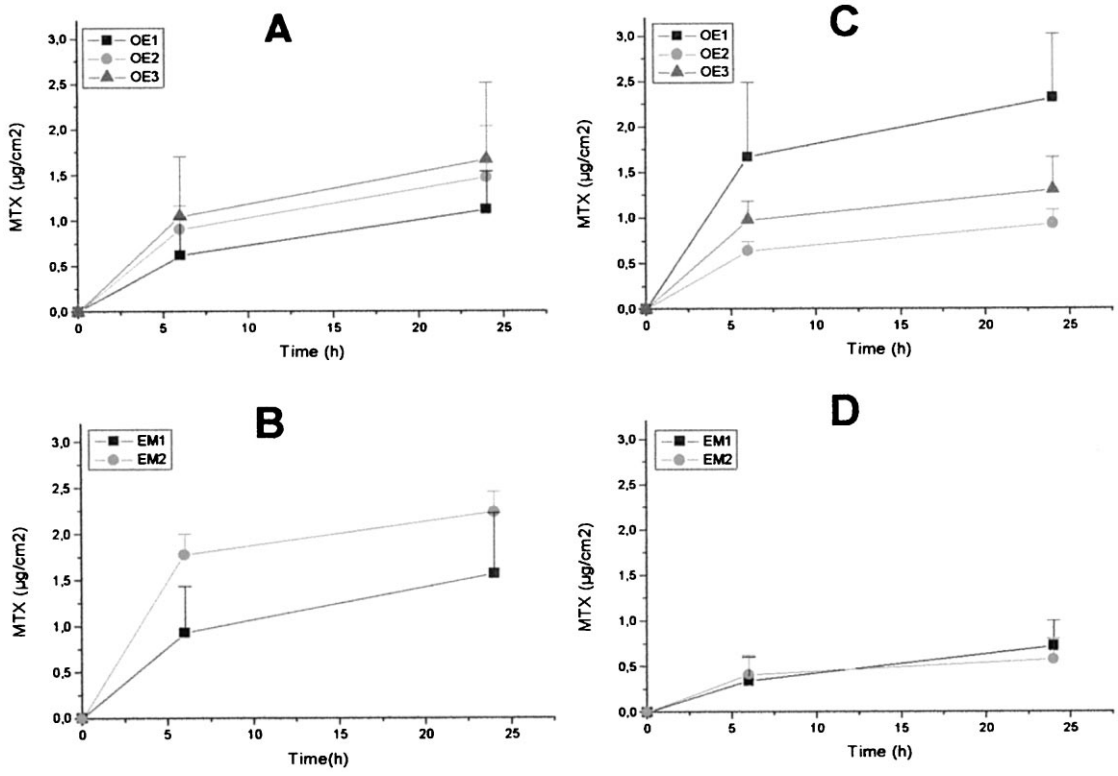

Fig. 1. Summarized results of the assays of passive delivery of MTX from microemulsions: amounts of MTX in the receptor solution after 6 and 24 h (µg cm^{-2}) (37°C). MTX concentrations in each system are shown in Table 4. (A) System I, 80% MTX saturation; (B) system II, 80% MTX saturation; (C) system I, 50% MTX saturation; (D) system II, 50% MTX saturation. Values shown are means ± SD for six assays.

wide range while holding all other factors constant. The EM system is particularly problematic in this regard: it shows two small stability zones in the o/w/surfactant phase diagram, one in which w/o microemulsions are formed, and another (very small) in which o/w microemulsions are formed.

Some previous studies (Osborne et al., 1988, 1991), have found that drug release from microemulsions is dependent on their proportional composition, and notably on their proportional water content. In the present study, we observed significant effects of composition for the OE-50% systems, but not for OE-80% systems or the EM systems.

Pairwise comparisons for each formulation (i.e. OE1-80% vs OE1-50%, etc.) indicate that the amount of MTX crossing the skin in most cases differs considerably depending on MTX saturation. This again points to the importance of MTX concentration in determining passive diffusion from microemulsions.

Several studies have compared o/w and w/o microemulsions with other vehicles for the transdermal administration of medicines, such as lotions, suspensions, gels or emulsions (Linn et al., 1990; Gasco et al., 1991; Trotta and Gasco, 1997). It has been observed that microemulsions have a greater capacity to release drugs towards the skin. This may be due to the fact that drugs contained in microemulsions are in dissolved or suspended form, so that their absorption is faster and more effective. We found that passive transdermal delivery of MTX from microemulsions was more effective than passive transdermal delivery from a simple solution (Weintein et al., 1989; Álvarez-

Figueroa et al., 2000), but less effective than iontophoretic transdermal delivery from a simple solution (Álvarez-Figueroa et al., 2000). In the case of MTX, the greater effectiveness of iontophoretic delivery from solutions than of passive delivery from microemulsions may be attributable to the lower solubility of MTX in microemulsions than in simple aqueous solution.

A previous study of the passive delivery of MTX from W/W microemulsions across nude mouse skin obtained a delivery rate of about 4–5 $\mu g\ cm^{-2}\ h^{-1}$ (Trotta et al., 1996). This is markedly higher than the rate obtained in the present study, presumably because the diffusion of MTX (a hydrosoluble compound) is favoured when it is incorporated into aqueous vehicles. In the present case, and although the differences were not always statistically significant, we observed more effective delivery of MTX from the microemulsion with aqueous external phase (system I).

The amounts of MTX remaining in the skin at the end of the assay are shown in Table 5. The observed behaviour was similar to that obtained for amounts of MTX entering the receptor solution. In the case of microemulsions with MTX at 80% saturation, we did not detect statistically significant between-formulation differences in delivery to skin. In the case of microemulsions with MTX at 50% saturation, we detected a number of statistically significant between-formulation differences in delivery to skin. For all formulations, the amount of MTX remaining in the skin was significantly higher when MTX content was 50% saturation than when MTX content was 80% saturation.

The amounts of MTX remaining in the skin in these experiments are similar to the amounts remaining in the skin in the assays of iontophoretic delivery from hydrogels.

Table 5
Summarized results of the assays of passive delivery of MTX from microemulsions: amounts of MTX remaining in the skin after 24 h ($\mu g\ cm^{-2}$) (37°C)[a]

Microemulsion	MTX saturation (%)	MTX in the skin ($\mu g\ cm^{-2}$)
OE1	80	0.61 ± 0.07
OE1	50	0.86 ± 0.25
OE2	80	0.91 ± 0.22
OE2	50	2.11 ± 0.83
OE3	80	1.11 ± 0.56
OE3	50	2.19 ± 0.44
EM1	80	0.92 ± 0.38
EM1	50	2.91 ± 0.39
EM2	80	1.12 ± 0.31
EM2	50	2.21 ± 0.72

[a] Values shown are mean ± SD for six assays.

4. Conclusions

Both iontophoretic transdermal delivery of MTX from hydrogels, and passive transdermal delivery from microemulsions were more effective than passive transdermal diffusion from MTX solutions (Weintein et al., 1989; Álvarez-Figueroa et al., 2000). The effectiveness of iontophoretic delivery from hydrogels was not affected by the type of hydrogel used, or by MTX concentration in the loading solution. The effectiveness of passive delivery from microemulsions, showed some relationship to MTX concentration, but appeared not to be affected by microemulsion composition or phase proportion. In all cases detectable amounts of MTX remained in the skin at the end of the assay. The increased effectiveness of MTX delivery observed in the present study suggests that topical administration of this drug may be feasible, but since the optimal skin concentrations of MTX for treatment of psoriasis are not known, we cannot confirm that the concentrations reached will be effective. However, both hydrogels and microemulsions are versatile systems whose composition and properties can be readily modified. Optimization of system properties might therefore improve their delivery of MTX to the skin.

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