

A study of oleic acid oily base for the tropical delivery of dexamethasone microemulsion formulations

M Bajpai, P K Sharma, A Mittal¹

Departments of Pharmacy, Raj Kumar Goel Institute of Technology, ¹Pharmaceutical Sciences, Shree Ganpati Institute of Technology, Ghaziabad, Uttar Pradesh, India

Dexamethasone Microemulsion systems composed of Water, oleic acid; Tween 80 and Isopropyl alcohol were investigated as potential drug delivery vehicles. Pseudo-ternary phase diagram was constructed at room temperature by titration, and the oil-to-surfactant/co-surfactant mass ratios (O/SC) that exhibit the maximum in the solubilization of water were found. Microemulsion formulations were evaluated for pseudo ternary phase study, Globule size, thermal stability, centrifugation stress testing, specific gravity, pH study, in vitro release on rat abdominal skin. The permeation data showed that microemulsion formulations increased dexamethasone flux 200–400 fold over the control, but permeability coefficients were decreased by 4 times. The superior transdermal flux of dexamethasone was due to 1000 fold improvement in solubilization of dexamethasone by microemulsions using lecithin. It can be concluded from the study that the dexamethasone microemulsions can be potentially used for improved topical drug delivery.

Key words: Dexamethasone, lecithin, microemulsion, oleic acid, permeation enhancement, transdermal delivery

INTRODUCTION

Most of the synthetic drugs that are being developed are lipophilic in nature and have poor water/ aqueous solubility thereby posing problems in their formulation into delivery system. A long problem to the pharmaceutical industry and a good deal of research could be done in this area. Newer and novel drug delivery technologies developed in recent years for these type of drugs and one of the technology is microemulsions. Microemulsions are thermodynamically stable isotropic systems in which two immiscible liquids are mixed to form a single phase by means of an appropriate surfactant or its mixture. These are homogenous systems of low viscosity that can be prepared over a wide range of surfactant concentrations and oil-to-water ratios. Microemulsions are frequently called solubilized systems because on a macroscopic basis they seem to behave as true solutions. Hoar and Schulman introduced the word microemulsion (ME), which they defined as a transparent solution obtained

by titrating a normal coarse emulsion with medium-chain alcohols.^[1] The presence of surfactant and co-surfactant in the system lowers the interfacial tension. Therefore, the microemulsion is thermodynamically stable and forms spontaneously, with an average droplet diameter of 10 to 140 nm.^[2,3] Microemulsion contain droplets of oil in water phase (o/w) or droplets of water in oil phase (w/o) with diameter of about 10-200 nm and the volume fraction of dispersed phase vary from 0.2 to 0.5. A microemulsion one of the pharmaceutical interests for new drug delivery system is normally composed of oil, water surfactant and co-surfactant.^[4] Microemulsions have the ability to deliver larger amounts of water and topically applied agents into the skin than water alone or other traditional vehicles, because they act as a better reservoir for a poorly soluble drug through their capacity for enhanced solubilization.^[5,6] The phase diagram characterizes microemulsions regions as the amount of oil, water and surfactant-co-surfactant mixture can be determined by plotting pseudo-ternary phase diagrams. The phase diagram also reveals the various other regions like micellar region, reverse micellar area, macroscopic emulsion o/w or w/o or bicontinuous laminar region.^[7]

Due to their specific properties and numerous advantages, microemulsions are promising systems for topical drug delivery. They can increase water

Address for correspondence:

Dr. P. K. Sharma, Department of Pharmacy,
Raj Kumar Goel Institute of Technology,
5 Km Stone, Meerut Road, Ghaziabad, Uttar Pradesh, India.
E-mail: pankajsharma_79@indiatimes.com

DOI: 10.4103/0973-8398.56299

solubility of the drug and enhance drug absorption into the skin. Microemulsion systems have extensive interfacial, aqueous and oily domains, so are capable of dissolving considerable quantities of oil soluble, water-soluble and amphiphilic materials. They form spontaneously without high shear equipment or significant energy input, and their microstructure are independent of the order of addition of the excipients. Optical transparency and low viscosity of microemulsions ensure their good appearance, easy to handle, pack and long shelf life.^[8] Microemulsion systems represent a promising prospect for the development of formulation suitable for the incorporation of poorly water-soluble drugs due to high solubilization capacity as well as the potential for enhanced absorption. In addition the solution like feature of microemulsion could provide advantages such as sprayability and dose uniformity. In recent years microemulsion has been extensively studied for transdermal, parenteral and oral delivery of drugs.^[9,10] In addition to increased physical stability, microemulsions often function as super solvent for certain compounds. Thus these clear fluids may dramatically increase the solubility/solubilization of poorly soluble drugs. While microemulsions have significant potential as drug delivery vehicles only few well-characterized surfactant systems have been systemically studied.^[11] A topical treatment of several diseases is often limited by the poor percutaneous permeation through the human skin. For this reason the realization of topical formulations with are able to improve the percutaneous permeation of adrenocorticoid steroid (Anti inflammatory) drugs can be of particular importance for the success of topical therapeutic approaches.^[12,13] The common method to improve drug permeation through the skin is to use permeation enhancer's i.e. organic solvents and fatty acids. Penetration enhancers can bring changes in the structure of skin lipids and alter the skin barrier function.^[14]

MATERIALS AND METHODS

Materials

Dexamethasone was a gift from Arbro Pharmaceuticals (Delhi, India) and other excipients and reagents were purchased from the following manufacturers: Oleic acid (CDH Laboratory Reagent, New Delhi, India), Tween 80 (CDH Laboratory Reagent), isopropyl alcohol (Qualigens) and lecithin from Acros Chemicals. The model drug was sieved and the 74-44 μ m fraction (mesh# 200-mesh# 325) was used in the studies.

Methods

Preparation of the skin

A number of membranes can be used for performing *in vitro* permeation studies. Although human cadaver skin is the best fit, membranes like rat abdomen skin have been used for the permeation studies in various studies. For the permeation studies, albino rats were selected because of their easy availability. The Institutional Animal Ethical Care Committee approved the protocol for use of rat abdomen skin.

Rats were sacrificed by chloroform vapors. The dorsal skin of the animal was shaved and the skin in full thickness was removed surgically. Excised skin from the rat abdomen was dipped into hot water at 60°C for 60s. The subcutaneous fat was removed and the skin was washed with water. The skin samples were examined for integrity and placed in a refrigerator at 4°C overnight before use. For further use, the skin samples were stored at -20°C in a deep freezer.

In vitro permeation study

In vitro skin permeation across the rat abdomen skin was conducted using a Franz diffusion cell. The excised skin was mounted on the diffusion cell with the stratum corneum side facing toward the donor compartment. The area of the diffusion cell used for all *in vitro* permeation studies was 1.767 cm² and the capacity of the receiver compartment was 15.0ml. The skin was equilibrated for 1h with the receiver medium. A blank sample (1.0ml) was withdrawn from the receptor compartment and analyzed to ensure any residual absorbance. The receptor medium (phosphate buffer pH6.8) was replaced with the fresh medium. The receptor chamber was thermostated at 37 \pm 2°C and a magnetic stirrer was used to stir the solution in the receptor chamber continuously.

Three milliliters of the microemulsion formulation containing a specified quantity (780 μ g/cm³-1000 μ g/cm³) of Dexamethasone was filled in the donor chamber. Samples (1.0 ml) were withdrawn from the receptor compartment for 24h at the interval of 4h and the drug content was analyzed by the UV spectrophotometry method at λ_{max} 240nm using phosphate buffer of pH 6.8 as a blank. The receptor volume was immediately replaced with an equal amount of receptor medium. The sampling port and donor chamber were covered by an aluminum foil to prevent evaporation of the receptor medium.

Preparation of microemulsion formulations

The oil (oleic acid) and the water phases were combined in various ratios with the surfactant (Tween 80) in which the cosurfactant (Isopropyl alcohol) was added gradually with magnetic stirring at room temperature until the system was transparent. Microemulsions were allowed to equilibrate with gentle magnetic stirring for 30 min. The excess amount of Dexamethasone was added to microemulsion and allowed to equilibrate in the mixer under constant mixing for 4 days at room temperature. The saturated solution was then filtered through 0.45 μ m Millipore [Table 1].

Determination of microemulsion type

In the determination of the type of microemulsion, various methods were used. The adopted method for the microemulsion is the electroconductivity test. The O/W type microemulsions showed electrical conductivity while the vehicles that did not give a conductivity value were considered a W/O type microemulsion system. All

Table 1: Quantity used of various o/w microemulsions of oleic acid

| Ingredients | ME-6 % | ME-7 % | ME-8 % | ME-9 % | ME-10 % |
|--|-----------|-----------|-----------|-----------|------------|
| Oleic acid | 10 | 8.84 | 8.47 | 9.52 | 9.09 |
| Tween 80 | 20 | 31.26 | 39.30 | 18.36 | 9.09 |
| Iso propyl alcohol | 20 | 15.63 | 9.825 | 24.48 | 36.36 |
| Water | 50 | 44.24 | 42.37 | 47.61 | 45.45 |
| Ratio (surfactant/ cosurfactant) | 1:1 | 2:1 | 4:1 | 3:4 | 1:4 |
| Oil/water ratio | 1:5 | 1:5 | 1:5 | 1:5 | 1:5 |
| Drug content ($\mu\text{g}/\text{cm}^3$) | 1000 | 833.33 | 846.66 | 953.33 | 910 |

Factors affecting the microemulsion stability

the microemulsion formulations showed that they contain oil in the internal phase and water in the external phase.

Determination of the globule size

The globule size of the microemulsion formulation was determined by JDS Quasi Elastic Light Scattering, Uniphase, US Instruments. Through the light scattering method, the size determination is much easier than by the photomicroscope method.

Determination of thermal stability

Twenty milliliters of drug-loaded microemulsions were stored in a 25 ml transparent borosil volumetric container at three different temperatures, i.e. 4°, 25° and 40°C, 1°C in BOD for a period of 1 month. Samples were periodically removed for visual inspection to observe any physical changes like loss of clarity, coalescence and turbidity, etc. Also, the samples were observed for the determination of loss of aqueous phase that is an essential part of the microemulsion stability.

Centrifugation stress testing

Centrifugation stress of 5000 and 10,000 rpm for 30 min were applied in order to assess the physical instabilities by a Remi centrifuge, like phase separation, phase inversion, aggregation, creaming and cracking of the microemulsion formulations. Previously thermally tested 2.5 ml formulation was taken in centrifuge sample tubes and placed in the centrifuge basket at a well-balanced equilibrium position at ambient temperature conditions.

Specific gravity testing at 28°C

To determine the specific gravity, a capillary gravity bottle method was used. Washed and dried, the precaution was necessary during the drying of the gravity bottle as a little amount of moisture could increase the errors in the data of the specific gravity of the samples.

pH of the microemulsions

The microemulsion samples were taken into the sample tubes and a μ pH meter was used to determine the pH of the different samples as the pH of the formulation is not the only factor and that the stability of the microemulsions also

imparts a role to alter the bioavailability of the drug through microemulsion at the site of permeation.

RESULTS AND DISCUSSION

Determination of the type of microemulsion

Emulsion type, whether w/o or o/w type of microemulsion, was determined by electrical conductivity measurement. For the determination of the type of emulsion, 1% w/v solution of sodium chloride was used as the aqueous phase instead of distilled water. Twenty-five milliliters of the drug-loaded microemulsion base was taken in a beaker at ambient temperature conditions. A conductimetric electrode was dipped into the beaker containing microemulsion and connected to an electric switch and the conductivity value was observed after a dynamic condition were set up within 10 min.

The o/w type of microemulsions showed electrical conductivity while the vehicles that did not give a conductivity value were considered to be the w/o type of microemulsion system. All the microemulsion formulations showed that they contain oil in the internal phase and water in the external phase.

Globule size determination

The globule size of the microemulsion formulation was determined by JDS Quasi Elastic Light Scattering, Uniphase, US Instruments. Twenty milliliters of the microemulsion formulation previously equilibrated to 30°C was filled in a 25 ml capacity transparent, borosilicate glass tube. The instrument took an hour to set up for initialization. The glass tube with the highly clean outer surface with complete removal of the solvent/oil/dirt was placed in the space provided for the laser beam to incident. Again, a 10-min interval was taken to read using the system software. Input of the nature of the solvent and solvent data were required to carry out the globule size analysis [Table 2, Figures 1-10].

Thermal stability

Twenty milliliters of drug-loaded microemulsions (either castor oil or oleic acid of any surfactant/cosurfactant ratio) were stored in a 25 ml transparent borosil volumetric container at three different temperatures, i.e. 4, 25 and 40°C, 1°C in BOD for a period of 1 month. Samples were periodically removed for visual inspection to observe any physical changes like loss of clarity, coalescence and turbidity, etc [Table 3].

Centrifugation stress testing

Centrifugation stress of 5000 and 10,000 rpm for 30 min were applied in order to assess the physical instabilities by Remi centrifugation, like phase separation, phase inversion, aggregation, creaming and cracking of the microemulsion formulations. Previously thermally tested 2.5 ml formulation was taken in centrifuge quvettes and placed in the centrifuge basket at a well-balanced equilibrium position at ambient temperature conditions [Table 4].

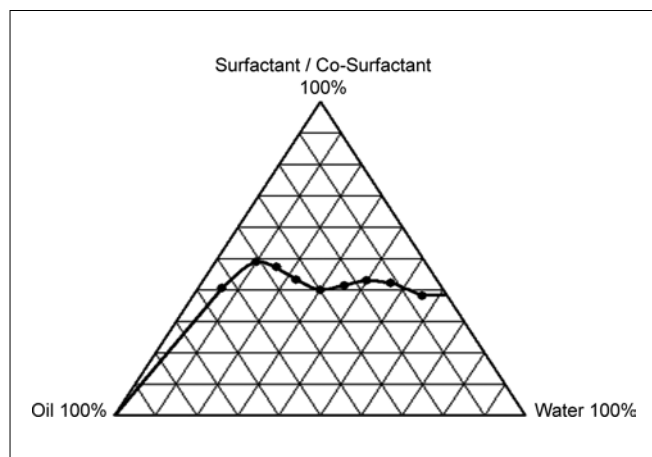


Figure 1: Phase equilibria of ME6

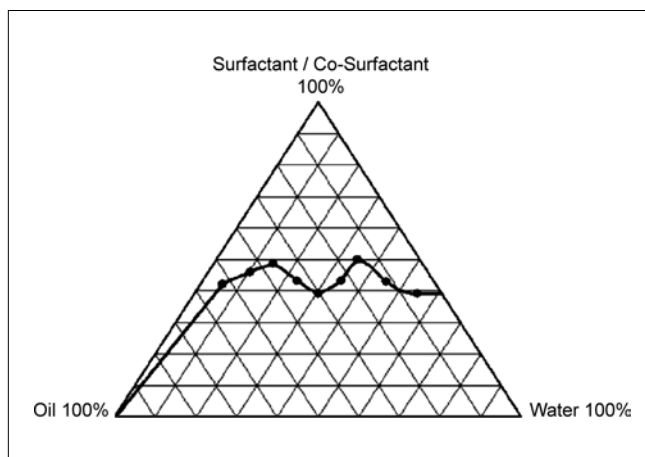


Figure 2: Phase equilibria of ME7

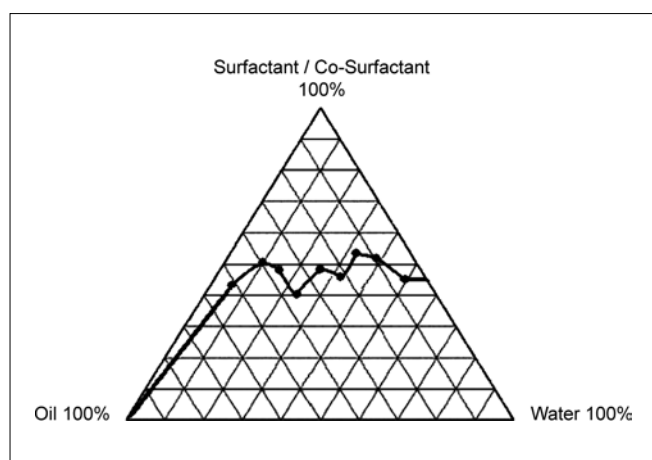


Figure 3: Phase equilibria of ME8

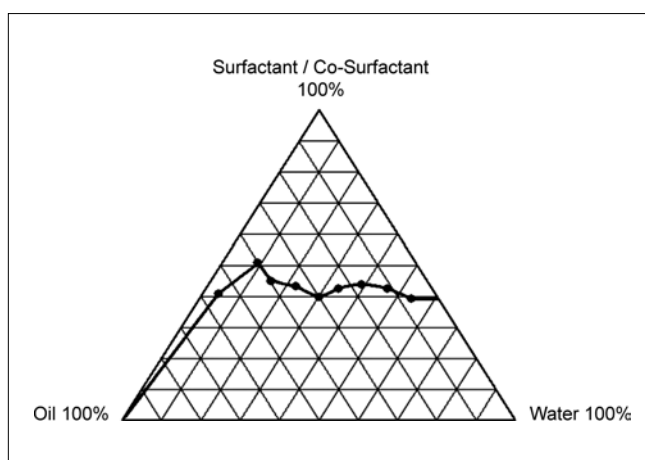


Figure 4: Phase equilibria of ME9

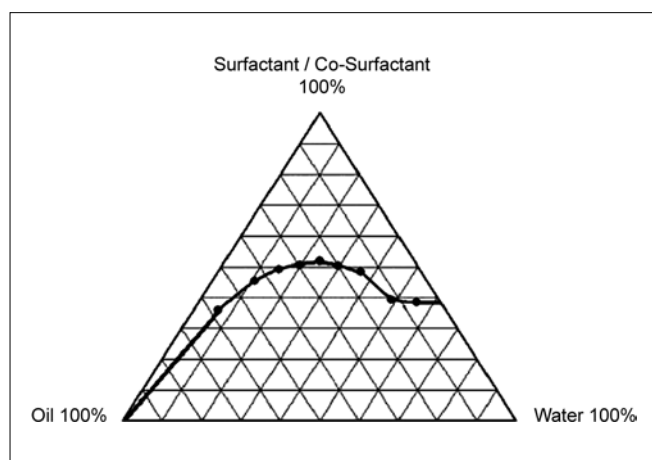


Figure 5: Phase equilibria of ME10

Specific gravity at 28°C

To determine the specific gravity, the capillary gravity bottle method was used. Washed and dried, an empty specific gravity bottle was weighed at room temperature (28°C). The bottle was filled with the microemulsion

Table 2: Globule size distribution of various microemulsion formulations

| Microemulsion formulations | Mean size (µm) | Polydispersity (SD/mean ²) | Skewness (third moment/SD ³) |
|----------------------------|----------------|--|--|
| ME-6 | 210.4 | 0.358 | -3.251 |
| ME-7 | 197.6 | 0.246 | -0.904 |
| ME-8 | 250.9 | 0.140 | -8.055 |
| ME-9 | 264.6 | 0.373 | -1.352 |
| ME-10 | 328.2 | 1.551 | -3.051 |

Table 3: Thermal stability of oleic acid microemulsions

| Oleic acid microemulsions | Storage condition (4°C) | Storage condition (25°C) | Storage condition (40°C) |
|---------------------------|-------------------------|--------------------------|--------------------------|
| ME-6 | √ | √ | X |
| ME-7 | √ | √ | √ |
| ME-8 | √ | √ | √ |
| ME-9 | √ | √ | √ |
| ME-10 | √ | √ | √ |

√, the microemulsions of the specific ratios at the particular conditions are stable. X, the microemulsions of the specific ratios at the particular conditions were not stable but were converted to the stable formulations by adding the specific amount of cosurfactant ratios

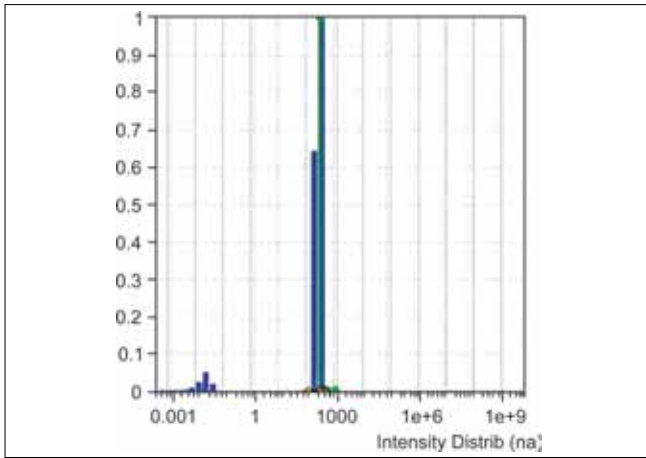


Figure 6: Intensity distrib (nm) ME6

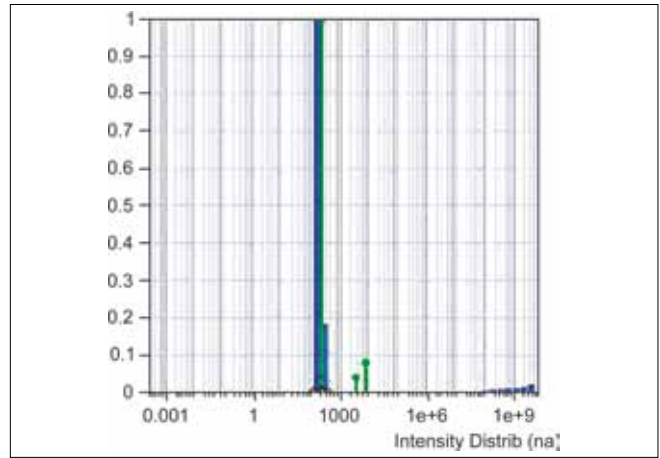


Figure 7: Intensity distrib (nm) ME7

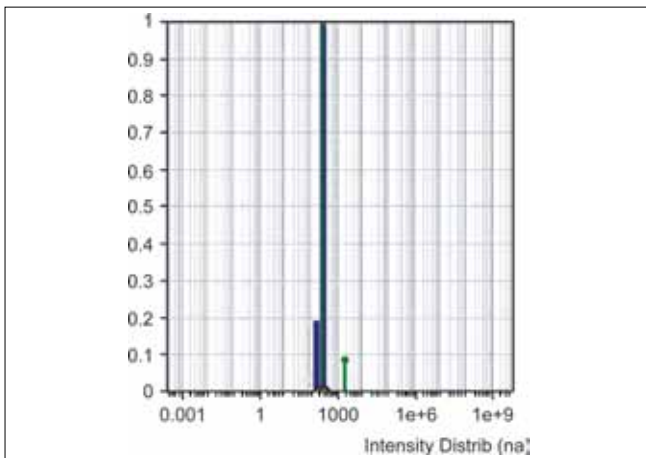


Figure 8: Intensity distrib (nm) ME8

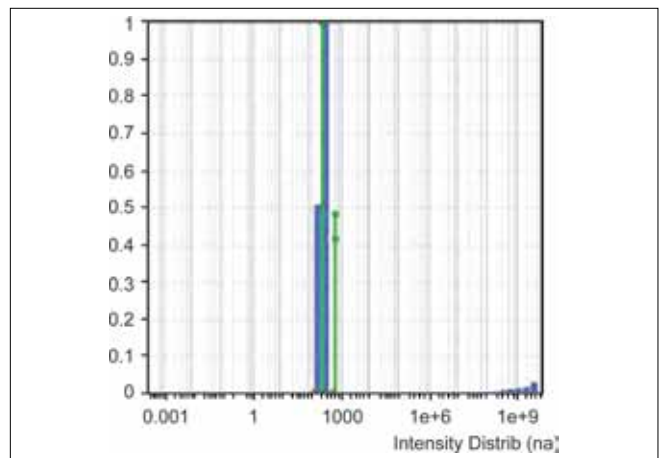


Figure 9: Intensity distrib (nm) ME9

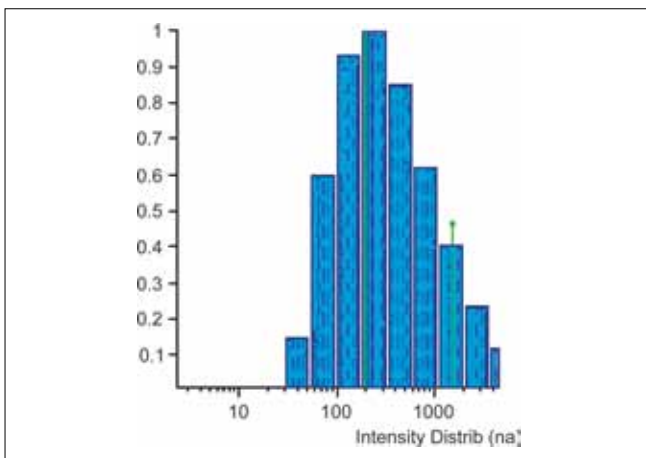


Figure 10: Intensity distrib (nm) ME10

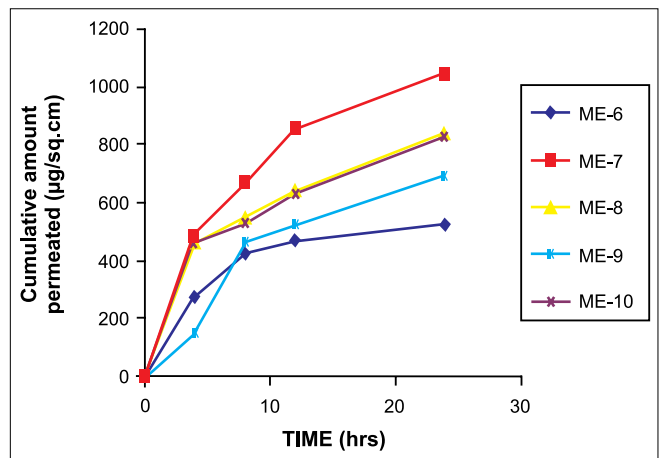


Figure 11: Cumulative amount permeable

and weighed [Table 5]. Specific gravity is calculated by following formula.

pH of the microemulsions

The microemulsion samples were taken into test tubes and using μ pH meter the pH was determined [Table 6].

In vitro permeation

During the optimization of the formulations, the *in vitro* permeation was calculated on the basis of the permeation through the rat abdomen skin. The formulation ME-7 has the highest permeation, i.e. 57.4% of the total drug incorporated in the formulation. On the basis of ME-7, the

optimized final formulation was designed ME-11 and ME-12, which show better results than the other formulations [Figures 11 and 12].

Formulations selected

On the basis of the cumulative drug permeated and the flux of the formulation, ME-11 and ME-12 were taken in which lecithin 0.1% was added to study its effect as a permeation enhancer [Table 7].

CONCLUSION

The present project was an attempt to achieve the possibility of preparation of topical microemulsions of dexamethasone with increased permeation through the skin. The microemulsion system is a promising approach for the topical delivery of dexamethasone. It represents an easy to manufacture

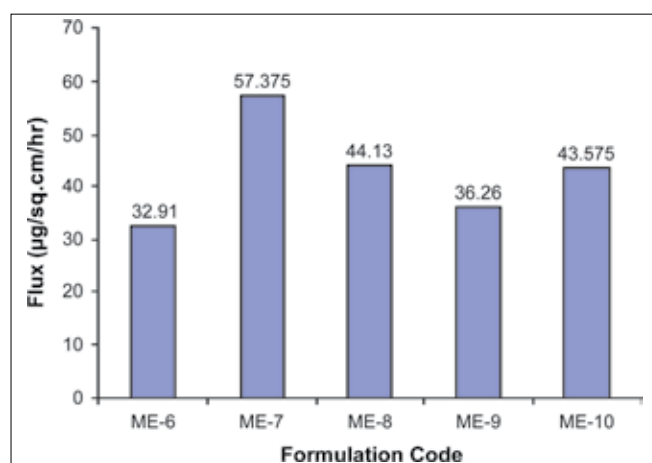


Figure 12: Graph of flux

Table 4: Centrifugation of various types of oleic acid microemulsions

| Oleic acid microemulsions | 5000 rpm | 10,000 rpm |
|---------------------------|----------|------------|
| ME-6 | √ | √ |
| ME-7 | √ | √ |
| ME-8 | √ | √ |
| ME-9 | √ | X |
| ME-10 | √ | √ |

√, the microemulsions of the specific ratios at the particular conditions are stable, no phase was separated. X, the microemulsions of the specific ratios at the particular conditions were not stable, phase was separated but was converted to the stable formulation by adding the specific surfactant/cosurfactant ratios

Table 5: The specific gravity of various oleic acid microemulsions

| Microemulsion formulations | Specific gravity |
|----------------------------|------------------|
| ME-6 | 1.027 |
| ME-7 | 1.0111 |
| ME-8 | 1.0071 |
| ME-9 | 1.0196 |
| ME-10 | 1.0067 |

thermodynamically stable system with improved topical availability of the drug and a transparent and elegant appearance.

- The stable microemulsion formulations of castor oil and oleic acid were successfully prepared with ease of fabrication from a pseudoternary phase diagram.
- Optimized formulations containing permeation enhancer (lecithin) successfully delivered the drug across the skin in 24 h with zero order kinetics.
- Rate and extent of drug lecithin microemulsion formulations were four times better than the gel formulation.

Thus, microemulsion formulations have been extensively used as research topics like vehicles for future candidates applied as topical as well as transdermal delivery systems.

ACKNOWLEDGEMENT

The authors are grateful to Arbro Pharmaceuticals, Delhi, India, for providing the gift samples of dexamethasone and the Department of Pharmacy, Raj Kumar Goel Institute of Technology, Uttar Pradesh Technical University, Lucknow, India, for providing the necessary research facilities.

REFERENCES

1. Eccleston GM. Encyclopedia of pharmaceutical technology. In: Swarbrick I, Boylar JC, editors. Vol. 9. Marcel Dekker; New York: 1992. p. 375-421.
2. Hiemenz PC. Principles of colloid and surface chemistry. New York: Marcel Dekker; 1986. p. 46.
3. Hiemenz PC, Rajagopalan R. Principles of colloid and surface chemistry. 3rd ed. New York: Marcel Dekker; 1997. p. 213.
4. Suryawanshi S, Singh SK, Gupta RC. A sensitive and selective HPLC/ESI-MS/MS assay for the simultaneous quantification of dexamethasone. J Chromatogr B Analyt Technol Biomed Life Sci 2006;830:54-63.
5. Škoviera F. Microemulsions as vehicles for transdermal Permeation of drugs. Acta Facult Pharm Univ Comenianae 2003;50:147-55.
6. Shaji J, Joshi V. Self-micro emulsifying drug delivery system (SMEDDS) for improving bioavailability of hydrophobic drugs and its potential

Table 6: The pH of the oleic acid microemulsions

| Microemulsion formulations | pH |
|----------------------------|------|
| ME-6 | 4.33 |
| ME-7 | 4.48 |
| ME-8 | 4.95 |
| ME-9 | 4.60 |
| ME-10 | 4.14 |

in vitro permeation

Table 7: Optimized formulation

| Ingredients | Quantity |
|----------------|-----------------------|
| Dexamethasone | 500 mg |
| Carbopol 934 | 1 g |
| Methyl paraben | 0.450 g |
| Propyl paraben | 0.050 g |
| Lecithin | 0.1% |
| Diethyl amine | Q.S. to adjust pH 7.0 |
| Water | Q.S. |

- to give sustain release dosage form. Indian Journal of Pharmaceutical Education 2005;39:130-5.
7. Jadhav KR, Shaikh IM, Ambade KW, Kadam VJ. Applications of microemulsion based drug delivery system. *Curr Drug Deliv* 2006;3:267-73.
 8. Derle DV, Sagar BS, Pimpale S. Microemulsion as a vehicle for transdermal permeation of nimesulide. *Ind J Phar Sci* 2006;68:622-5.
 9. Bali V, Bhavna, Ali M, Baboota S, Ali J. Potential of microemulsions in drug delivery and therapeutics: A patent review. *Recent Pat Drug Deliv Formul* 2008;2:136-44.
 10. Razdan R, Devrajan PV. Microemulsion-a review. *Indian Drugs* 2003;40:139-46.
 11. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev* 2000;45:89-121.
 12. Ranade VV. Drug delivery systems through transdermal delivery. *J Clin Pharmacol* 1991;31:401-18.
 13. Sintov AC, Shapiro L. New microemulsion vehicle facilitates precutaneous penetration *in vitro* and cutaneous drug bioavailability *in vivo*. *J Control Release* 2004;95:173-83.
 14. Chen H, Chang X, Weng T, Zhao X, Gao Z, Yang Y, *et al.* A study of microemulsion systems for transdermal delivery of triptolide. *J Control Release* 2004;98:427-36.

Source of Support: Nil, **Conflict of Interest:** None declared.

Author Help: Reference checking facility

The manuscript system (www.journalonweb.com) allows the authors to check and verify the accuracy and style of references. The tool checks the references with PubMed as per a predefined style. Authors are encouraged to use this facility, before submitting articles to the journal.

- The style as well as bibliographic elements should be 100% accurate, to help get the references verified from the system. Even a single spelling error or addition of issue number/month of publication will lead to an error when verifying the reference.
- Example of a correct style
Sheahan P, O'leary G, Lee G, Fitzgibbon J. Cystic cervical metastases: Incidence and diagnosis using fine needle aspiration biopsy. *Otolaryngol Head Neck Surg* 2002;127:294-8.
- Only the references from journals indexed in PubMed will be checked.
- Enter each reference in new line, without a serial number.
- Add up to a maximum of 15 references at a time.
- If the reference is correct for its bibliographic elements and punctuations, it will be shown as CORRECT and a link to the correct article in PubMed will be given.
- If any of the bibliographic elements are missing, incorrect or extra (such as issue number), it will be shown as INCORRECT and link to possible articles in PubMed will be given.