ORIGINAL ARTICLE

Using Inverted Microemulsions for Transdermal Application of Folic Acid

Jamal Alyoussef Alkrad[1,2], Sedeeq Mustafa Al-doori[1], Hussainyousef AlGhatm[1]

[1]Department of Pharmaceutical and Clinical Sciences, Faculty of Pharmacy, Isra University, PO Box 22 and 23, Amman, Jordan, [2]Department of Pharmaceutics, Faculty of Pharmacy, Al-Rasheed International University for Science and Technology, Damascus, Syria

Abstract

Objectives: This study aimed to improve and assess the transdermal of folic acid through the skin through non-ionic microemulsions (MEs). **Methods and Instruments:** Eight MEs were developed with different ratios of Span 20-to-Tween 80 and folic acid contents. These MEs were characterized for their droplet sizes, rheological properties. Besides, the encapsulation of folic acid insides the MEs was studied using Fourier-transform infrared spectroscopy. Furthermore, the flux of formulated folic acid in MEs was evaluated using Franz diffusion cell. **Results:** The MEs showed Newtonian viscosity and droplets sizes <200 nm. However, the droplets sizes increased with increasing Tween 80 and folic acid content. Furthermore, the flux of folic acid related to folic acid concentration. However, using MEs, a high flux of $11.47 \pm 6.9 \times 10^{-4}$ µg/cm^2 × h of folic acid through rat's skin could be achieved. **Conclusion:** The developed non-ionic MEs containing folic acid can be ideal carriers for administration of folic acid transdermally.

Key words: Folic acid, transdermal, microemulsions

INTRODUCTION

A low folate in pregnancy increases the risk of fetal neural tube defects.[1] Furthermore, its deficiency elevates plasma homocysteine, which is a risk factor for cardiovascular disease, stroke, and dementia.[2-4] These conditions can be reduced by folic acid supplementation. The bioavailability and bioaccessibility of natural or fortified food largely depends on several pre-absorptive and post-absorptive factors. Furthermore, folic acid has a short half-life of 1.5 ± 0.45 h.[5,6] Hence, folic acid is a good candidate for transdermal application. The skin has a unique structure. Besides its function as chemical and physical barrier, it permits transcellular, intercellular, or through appendage absorption of drugs under specific conditions.[7-10] This function allows application many drugs in controlled manner without losing their efficacy by the first pass effect, in gastric juice or by enzymatic digestion. Furthermore, this treatment is easy and self-applicable without suffering of side effects associated with oral application.[11-13] The transdermal drug bioavailability can be enhanced using the distinctive structure, nanosize, and rheological properties of fluid drug delivery systems such as microemulsions (MEs).[14] Non-ionic surfactants were used frequently in preparing ME for their low toxicity, penetration enhancing effect, and skin tolerability.[15-18]

Parade and Sirivat, 2016, studied the release of FA from the Zeolite Y/alginate hydrogel matrix and showed that FA diffused in controlled manner or by passive diffusion.[19] In another study, Kapoor *et al*. developed liposomes containing folic acid by thin-film hydration method. The *in vivo* topical application in rats of developed liposomes showed 11-fold increase in plasma folate within 2h, confirming systemic delivery through skin.[20]

This study aimed to develop MEs using non-ionic surfactants for transdermal application of folic acid.

Address for correspondence:
Jamal Alyoussef Alkrad,
Faculty of Pharmacy, Isra University,
PO Box 22 and 23, Amman, Jordan.
Phone: +91-962789696157.
Fax: 0096264711505.
E-mail: jamal.alkrad@iu.edu.jo

Received: 25-02-2018
Revised: 16-03-2019
Accepted: 23-03-2019

MATERIALS, INSTRUMENTS AND METHODS

Materials

Folic acid was purchased from AZ Chem group (China). Sorbitan monolaurate (Span® 20) and Polyoxyethylenesorbitan.mono-oleate (TWEEN® 80) were purchased from SIGMA (Lyon, France). Acetic acid and isopropyl myristate (IPM) were obtained from Merck (Darmstadt, Germany). Magnesium chloride was purchased from S D Fine-Chem Limited (India).

Instruments and Methods

MEs preparation

MEs were prepared by titration method.[21] Folic acid was dissolved in alkalized water (pH 10) using NaOH. 3 ml of IPM was added to 1 ml of prepared folic acid solution. Then, mixtures with definite ratio of Span 20:Tween 80 were added dropwise with continuous stirring over magnetic stirrer to the lipophilic and hydrophilic phases mixture until a clear ME was formed. The consumed surfactants volume was recorded. Amounts of folic acid either of 20 and 15 mg were used for each ratio in the developed MEs as tabulated in Table 1.

Pseudoternary phase diagrams of ME systems

A pseudoternary phase diagram was plotted to determine the proper ratios of the different components for preparing stable MEs.[21] Another three-phase diagram for MEs with folic acid was accomplished for testing the influence of folic acid on this area. Formulations were made using three components which are hydrophilic phase, lipophilic phase, and surfactants. Each phase is one face of the triangle. The formulations were made with fractions of the three components according to cross points which formed by plotting three parallel lines to the three faces of the triangle. More formulations were made between the cross points on the border of MEs area. After mixing, the clear and stable formulations were identified to be MEs.

Viscosity measurement

An electric rheometer made by Anton Paar, universal tool, model MCR 301 (Germany) was employed to determine the viscosity and rheological characteristics of MEs. Rheograms were plotted for the MEs with increasing shear force at 25°C on the bob and cup viscometers.

Droplet size measurement (Zeta potential measurement)

A laser Doppler electrophoresis was carried out on the MEs with a Zetasizer made by Malvern, UK, instrument which is capable of measuring particle size ranging between 0.3 nm and 10 µm. The sample was introduced into apparatus cell without dilution as the stability of MEs depends on the concentration. Samples were measured at temperature of 25°C without filtration.

Preparing rat's skin

The recommendation of guidance notes of organization for economic cooperation and development on dermal absorption was taken in consideration in evaluation the transdermal of folic acid using Franz diffusion cell and in preparing the skin.[22] Wistar male rats which were used for preparing rat's skin were purchased from University of Jordan and fertilized at Isra University.. All the measures were accomplished according to the NIH guidelines for the care and use of laboratory animals which were approved by the research committee of Isra University. For preparing the skin for Franz diffusion cell, the rats were shaved before executing the rats; then, the skin was peeled carefully to maintain its integrity. The adipose tissues were detached from the peeled skin and excised to small parts to fit with Franz diffusion cell surface area (with diameter a bit larger than 10 mm), then washed with buffer and stored in a deep freeze at a temperature below than −70°C.

Preparing the epidermis layer and fixing it in Franz Cell

Frozen rat's skin pieces were removed from the freezer and soaked in the 2 M solution of magnesium chloride overnight at 4°C; then, the epidermis layer was carefully peeled with forceps because it's thin layer and examined against the light

Table 1: The composition of different prepared MEs containing folic acid

MEs	IPM (ml)	Aqueous phase (ml)	FA (mg)	Surfactant type	Surfactant amount (ml)	FA concentration (mg/ml)
ME1	3	1	20	S20:T80 (4:2)	2.3	3.77
ME2	3	1	15	S20:T80 (4:2)	2.4	2.78
ME3	3	1	20	S20:T80 (3:2)	2.4	3.70
ME4	3	1	15	S20:T80 (3:2)	2.5	2.73
ME5	3	1	20	S20:T80 (3:3)	2.6	3.57
ME6	3	1	15	S20:T80 (3:3)	2.7	2.63
ME7	3	1	20	S20:T80 (2:4)	2.7	3.50
ME8	3	1	15	S20:T80 (2:4)	2.9	2.54

IPM: Isopropyl myristate, MEs: Microemulsions

for their integrity, then put on orifice of donor compartment with aid of large beaker filled with water (the upper surface of epidermis must be toward the donor compartment and the lower surface toward the accepter compartment).

Transdermal study of folic acid using Franz diffusion cell

Multi Franz diffusion cells apparatus made by Orchid scientific in India (10 mm diameter, 5 ml acceptor volume) was used in this study. The cells were adjusted at a temperature of 32 ± 0.1°C using circulatory water bath. The acceptor compartment was filled with 5 ml mixture of water and methanol (30:70). ME was distributed over the epidermis using an insulin syringe by a volume of 0.2 ml. Only ½ ml samples were withdrawn from the acceptor's orifice after 1, 3, 5, 7, and 24 h for analyzing transdermal folic acid using HPLC method. The withdrawn volume was replaced directly by the same volume of acceptor medium.

HPLC method and calibration curve for folic acid

A mobile phase of water: methanol (88:12) was pumped at flow rate of 1 ml/min. The water part was buffered using phosphate buffer using of 11.16 g potassium dihydrogen phosphate and 5.5 g dipotassium hydrogen phosphate for each 1 L of water. 5 ml of analyzed sample was injected into the mobile phase to separate using column of C18, 4.6 × 250 mm (Eclipse XDB 5). The detection was performed at wavelength of 280 nm.

Pharmacokinetic and statistical analysis

All related tests either the analysis or penetration studies are triplicated. Both the standard deviation and mean value are calculated. The passive diffusion is the way of transport across the skin. Origen program was used for statistical evaluation with confidence interval of 95%.

J_{ss} is the steady-state flux. It is calculated from the slope by plotting the penetrated amount per cm^2 (Q/A) against the time (t) as in Equations 1 and 2:[23]

$$Q/A = J_{ss} \times t \quad (1)$$

From Equation 1:

$$J_{ss} = \frac{Q}{A(t - t_{lag})} = K_p C_v \quad (2)$$

Where, K_p: Permeability coefficient and C_v: Vehicle concentration

From Equation 2:

$$K_p = \frac{K_{sc}\, D_{sc}}{h_{sc}} = \frac{J_{ss}}{C_v} \quad (3)$$

Where,
K_p: Permeability coefficient
D_{sc}: Diffusion coefficient through stratum corneum
K_{sc}: Partition coefficient between the excipient and the SC
A: Skin surface area
Q: The cumulative mass penetrating a membrane
C_s: The constant drug concentration in the donor solution

And h_{sc}: The thickness of the membrane or the diffusion path length or stratum corneum.

RESULTS

Pseudoternary phase diagram

Two pseudoternary phase diagram diagrams with and without folic were established [Figure 1] to find the best fractions of IPM, folic acid solution in water, and Span 20:Tween 80 (4:2) that form stable MEs. The MEs were formed for water fraction <0.4. However, MEs were appeared after folic acid addition with fractions >0.4. Furthermore, the area of clear MEs extended toward lesser fractions of the surfactants.

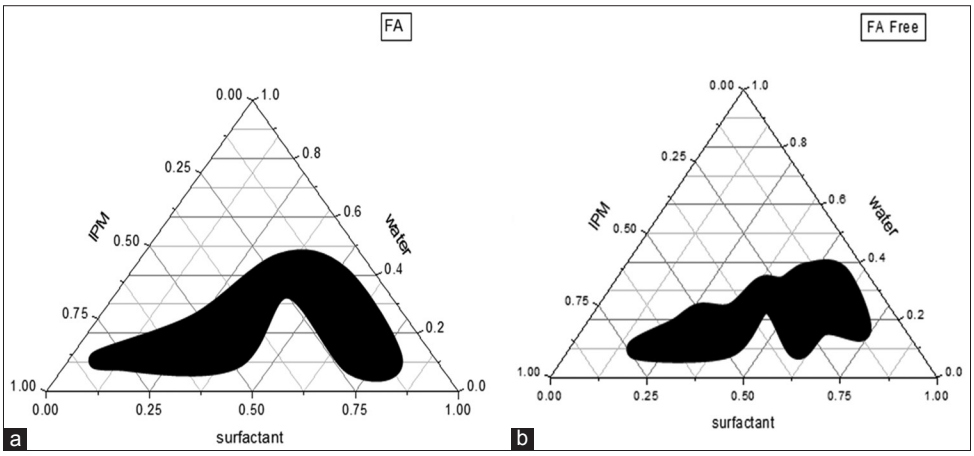

Figure 1: The pseudoternary phase diagram diagrams for the three components of; (a) IPM, water, and a mixture of Span 20:Tween 80 (4:2); (b) IPM, folic acid water solution, and a mixture of Span 20:Tween 80 (4:2)

Table 2: The measured droplet size, polydispersity index, and zeta potential for different formulated MEs containing folic acid

MEs	Droplet size (nm)	PDI	Zeta potential (mV)
ME1	124.0±6.6	0.84±0.06	−0.02±0.06
ME2	155.3±15.8	0.77±0.03	−0.04±0.03
ME3	144.3±9.3	0.98±0.04	−0.1±0.17
ME4	162.5±42.3	0.78±0.13	−0.1±0.10
ME5	177.9±26.5	0.88±0.12	−0.02±0.08
ME6	163.1±19.1	1±0	−0.09±0.05
ME7	215.1±45.3	0.97±0.05	0.22±0.29
ME8	10994.3±5311.5	0.68±0.54	−0.004±0.02

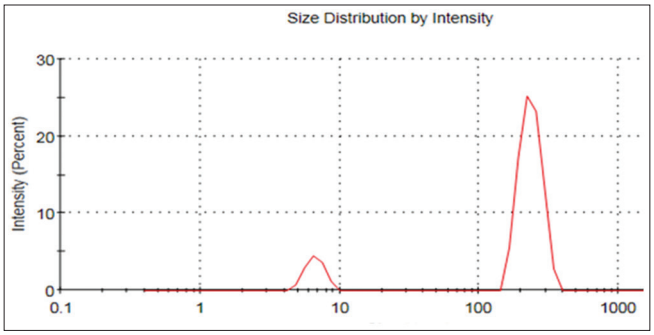

Figure 2: Zetasizer measurement of ME5

Droplet size measurements

The eight developed MEs containing folic acid were measured using Zetasizer to determine their droplet size using Zetasizer [Figure 2] and the results represented in Table 2.

The measurements show that the droplet sizes were <200 nm till ME7. Furthermore, the droplet sizes were increased with increasing Tween 80 proportion and decreasing folic acid content. In case of ME8 was unstable during measurement and showed droplet size near to 1000 nm. The zeta potentials were near to zero and negative.

Rheological properties

Measured viscosity was determined using bob and cup rheometer with increased share rate for the different systems and the results represented in Figure 3.

Figure 2 shows that the viscosity increased with increasing proportion of Tween 80 as well as increase folic acid content except the last system where the system with higher concentration had lower viscosity. However, the relationship between the shear rate and the viscosity forms straight line. Furthermore, the viscosity against the shear stress is constant. The results give evidence that systems have Newtonian characteristics. Consequently, all the systems exhibited ideal viscosity or Newtonian viscosity.

Studying of folic acid using Fourier-transform infrared (FTIR) spectroscopy

FTIR spectroscopy was used for studying the encapsulation of folic acid in the microemulsions droplets. The spectra of ME containing folic acid, free folic acid, and folic acid are represented in Figure 4.

The spectra of ME with and without folic acid were similar. Furthermore, the bands of the spectrum of folic acid did not appear in the spectrum of ME containing folic acid. Hence, folic acid was encapsulated in the surfactant in the inner phase.

HPLC method and transdermal evaluation using Franz diffusion cell for folic acid

A calibration curve for concentrations between 0.01 and 0.08 mg/ml was constructed to determine the amount of folic acid that penetrated through the skin to the acceptor using HPLC method. The calibration showed a linearity of 99.8% and regression standard deviation of 5.3×10^{-5}. The detection limit was below than 0.08 mg/ml (the lowest concentration in the calibration curve) as the signal height ratio of 0.08 mg/ml of the folic to the noise markedly >9.[24] The recovery using this HPLC method was 98% ± 1.4 folic acid of the contained amount in 0.5 ml of the MEs. However, Franz diffusion cell was used for the transdermal permeability of FA through shaved rat's skin over 24 h. The penetrated FA was collected by withdrawing 0.5 ml from the acceptor and analyzing it by HPLC. Penetrated FA amounts per cm^2 were measured over 24 h and cumulative measured amount per cm^2 plotted against the time [Figure 5].

The flux value was estimated from the slope at the steady state for the cumulative penetrated amounts against the time [Figure 6] and the results tabulated in Table 3.

The results show that as the concentration is high in the ME the flux increases. Furthermore, the flux decreased with increasing Tween 80 fraction which accompanied with

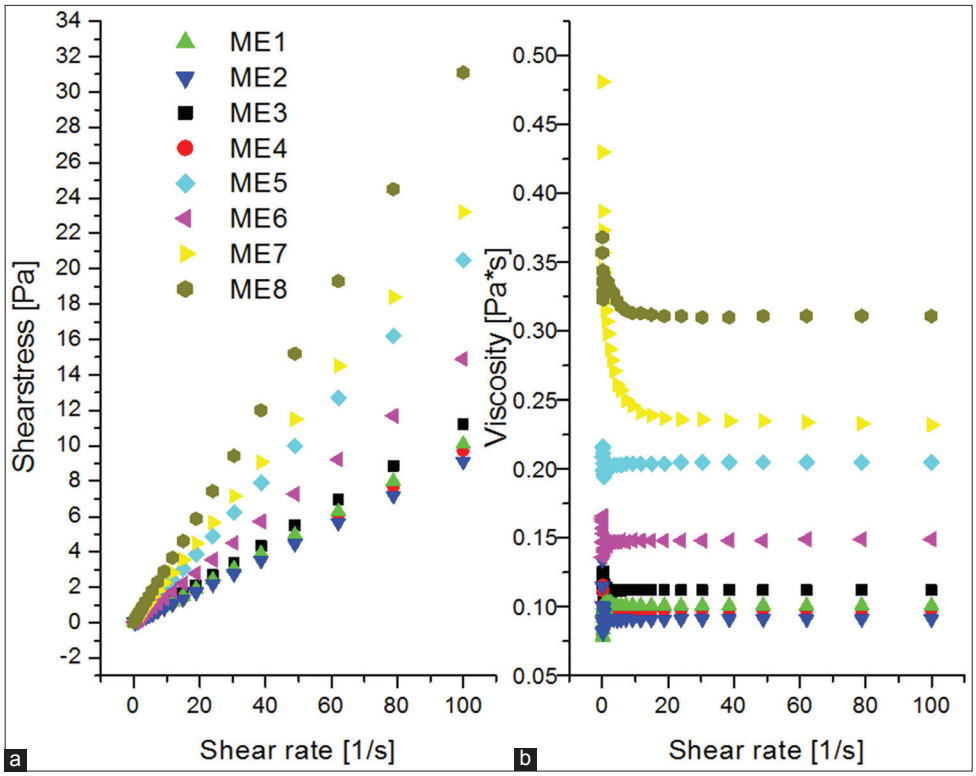

Figure 3: (a-b) The rheograms of different developed microemulsions containing folic acid. Shear rate against shear stress

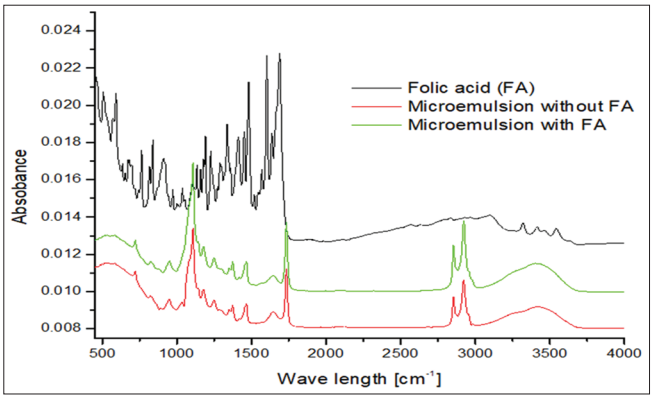

Figure 4: Fourier transform infrared spectra of folic acid as powder, ME with and without folic acid

increasing in the droplet sizes and the viscosity. However, ME ME1 [Table 3] which has the highest concentration of folic acid showed the highest flux value. However, the transdermal was rapid and the formulations did not show lag time.

DISCUSSION

MEs were prepared with two concentrations of folic acid for each ratio of Span 20 and Tween 80. The first group of ME contains 20 mg and the second contains 15 mg of folic acid. The ratio of Span 20 and Tween 80 was decrease toward decrease fraction of span 20 and at the same time increased faction of Tween 80. The first ME which was stabilized using Span 20:Tween 80 at ratio of 4:2 consumed the least surfactant amount. However, the consumed surfactant amount increased with decreasing folic acid content. Furthermore, the consumed surfactant amount was increased with increasing Tween 80's fractions. This may due to increasing the droplet size as shown in Table 3 which increases the interfacial area and increases the required surfactant amount for stabilizing the MEs.[25] In spite of the viscosity of Span 20 is higher than Tween 80, the viscosity increased with increasing Tween 80 fractions, may due to increasing the interaction between Tween 80 as hydrophilic molecule and the lipophilic phase. However, decrease amount of IPM <3 ml accompanied with gel forming. Using FTIR technic could be proved the presence of folic acid inside the droplet and the absent of them in the outer phase.

The increase in droplet size with increasing Tween 80 fractions and reduction Span 20 at the same time and decrease folic acid content may relate to increasing the greater molecule structure of Tween in comparison to Span 20, increasing interfacial tension with decreasing folic acid content or the activity of surfactants mixture with change in the ratio.[26]

The Gibbs adsorption isotherm [Equation 4] relates the surface tension (γ) to the amount of solute adsorbed at the interface (Γ):[25]

$$\tilde{A} = \frac{a}{RT} \cdot \frac{d\gamma}{da} \quad (4)$$

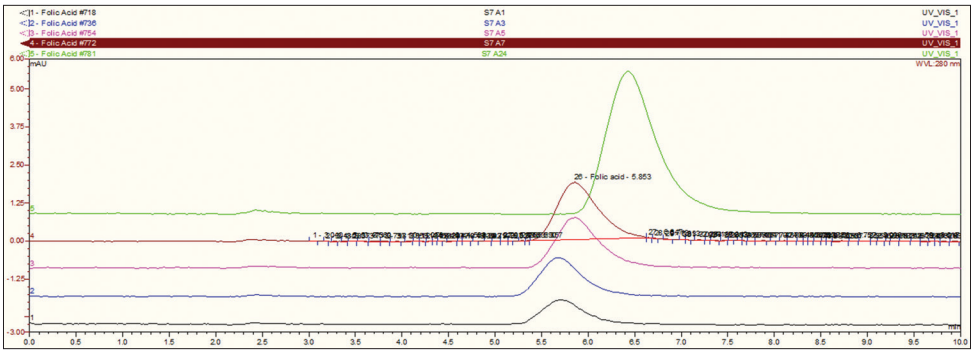

Figure 5: HPLC chromatogram of transdermal of folic acid between 1 and 24 h

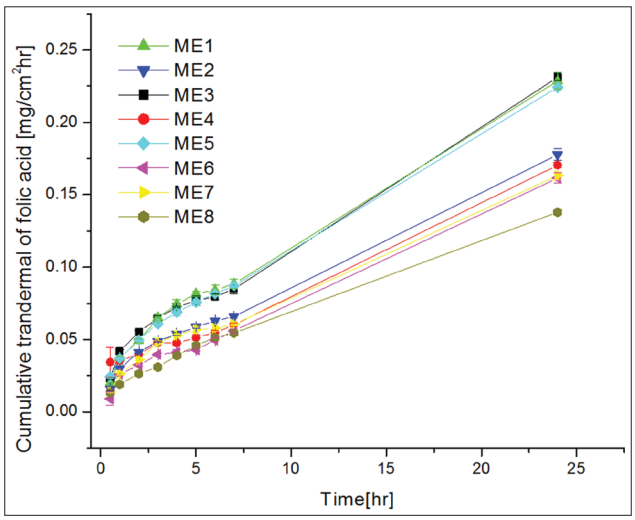

Figure 6: Transdermal profiles of folic acid using different formulations through rat's skin

Table 3: The flux of different formulated MEs containing folic acid through rat's skin using Franz diffusion cell

MEs	Conc. (mg/ml)	Flux (Jss) μg/cm² × h
ME1	3.77	$11.47\pm6.9\times10^{-4}$
ME2	2.78	$6.4\pm1.52\times10^{-4}$
ME3	3.70	$9.63\pm6.7\times10^{-4}$
ME4	2.73	$6.21\pm1.57\times10^{-4}$
ME5	3.57	$8.62\pm6.67\times10^{-4}$
ME6	2.63	$6.07\pm2.55\times10^{-4}$
ME7	3.5	$7.95\pm1.5\times10^{-4}$
ME8	2.54	$5.15\pm1.6\times10^{-4}$

MEs: Microemulsions

Where, *a*: the activity, *R*: gas constant; and *T*: absolute temperature

However, the developed containing folic acid formulations comply with MEs regarding their droplet sizes and rheological properties.[27]

As the penetration of the drug through the skin flows Fick's law,[23] the flux of folic acid using MEs was proportional to the concentration. However, the increase in the droplets sizes with increasing Tween 80 in the surfactant mixture decreased the flux of folic acid through the skin.

CONCLUSION

Using non-ionic surfactants were possible to develop new MEs containing folic acid able to transport folic acid through the skin. These MEs had colloidal characteristics regarding their droplet size, transparency, and rheological properties and suitable for transdermal application.

REFERENCES

1. Czeizel AE, Dudás I. Prevention of first occurrence of neural-tube defects by periconceptual vitamin supplementation. N Engl J Med 1992;327:1832-5.
2. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: Evidence on causality from a meta-analysis. BMJ 2002;325:1202-8.
3. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani A. Homocysteine and stroke: Evidence on a causal link from Mendelian randomisation. Lancet 2005;365:224-32.
4. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, *et al*. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476-83.
5. Saini RK, Nile SH, Keum YS. Folates: Chemistry, analysis, occurrence, biofortification and bioavailability. Food Res Int 2016;89:1-13.
6. Loew D, Eberhardt A, Heseker H, Kübler W. Plasma kinetics and elimination of folic acid. Klin Wochenschr 1987;65:520-4.
7. Bergstresser PR, Costner MI. Anatomy and physiology. In: Bolognia JL, Jorizzo JL, Rapini RP, editors. Dermatology. 2nd ed. London: Elsevier Science; 2008. p. 25-35.
8. Archer CB. Functions of the skin. In: Burns DA, Breathnach SM, Cox NH, Griffiths CE, editors. Rook's Textbook of Dermatology. 8th ed. Oxford, UK: Wiley-Blackwell; 2010. p. 401-11.
9. Baroni A, Buommino E, De Gregorio V, Ruocco E, Ruocco V, Wolf R. Structure and function of the

epidermis related to barrier properties. Clin Dermatol 2012;30:257-62.
10. Flynn GL, Stewart B. Percutaneous drug penetration: Choosing candidates for transdermal development. Drug Dev Res 1988;13:169-85.
11. Shingade GM, Quazi A, Sabale PM, Grampurohit ND, Gadhave MV, Jadhav SL, *et al*. Review on: Recent transdermal drug delivery system. J Drug Deliv Ther 2012;2:748-65.
12. Sahu M, Bhowmick M, Kushwaha R, Rathi J. Medicated transdermal therapeutic systems: An updated overview. Int J Pharm Chem Biol Sci 2017;7:43-9.
13. Upadhyay G, Verma S, Parvez N, Sharma PK. Recent trends in transdermal drug delivery system a review. Adv Biol Res 2014;8:131-8.
14. Nastiti CM, Ponto T, Abd E, Grice JE, Benson HA, Roberts MS. Topical nano and microemulsions for skin delivery. Pharmaceutics 2017;9:37-62.
15. Lo JT, Lee TM, Chen BH. Nonionic microemulsions as solubilizers of hydrophobic drugs: Solubilization of paclitaxel. Materials 2016;9:761-74.
16. Cadogan SP, Hahn CJ, Rausch MH, Fröba AP. Study on the applicability of dynamic light scattering (DLS) to microemulsions including supercritical carbon dioxide-swollen micelles. J Colloid Interface Sci 2017;499:202-8.
17. Weber A, Stühn B. Structure and phase behavior of polymer loaded non-ionic and anionic microemulsions. J Chem Phys 2016;144:144903.
18. Laffleur F, Pschick S, Barthelmes J, Hauptstein S, Bernkop-Schnurch A. Impact of surfactants on skin penetration of dexpanthenol. Curr Drug Deliv 2018;15:351-6.
19. Paradee N, Sirivat A. Encapsulation of folic acid in zeolite y for controlled release via electric field. Mol Pharm 2016;13:155-62.
20. Kapoor MS, D'Souza A, Aibani N, Nair SS, Sandbhor P, Kumari D, *et al*. Stable liposome in cosmetic platforms for transdermal folic acid delivery for fortification and treatment of micronutrient deficiencies. Sci Rep 2018;8:16122-36.
21. Prapaporn B, Karen K, Anja G, Thomas R, Varaporn BJ. Characterization of microemulsion structures in the pseudoternary phase diagram of isopropyl palmitate/water/brij 97:1-butanol. Am Assoc Pharm Sci Tech 2006;7:99-104.
22. Guidance Notes On Dermal Absorption, OECD Environment, Health and Safety Publications, Series on Testing and Assessment, No. 156, ENV/JM/MONO; 2011. p. 36. Available form: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2011)36&doclanguage=en. [Last accessed on 2019 Mar 27].
23. Li CJ, Obata Y, Higashiyama K, Nagai T, Takayama K. Effect of 1-O-ethyl3-butylcyclohexanol on the skin permeation of drugs with different physicochemical characteristics. Int J Pharm 2003;259:193-8.
24. Council of Europe. European Pharmacopoeia. 4th.ed. Strasbourg: Council of Europe; 2003.
25. Butt HJ, Graf K, Kappl M. Thermodynamics of interfaces. In: Physical Chemistry of Surfaces. New York; Wiley: 2003. p. 26-41.
26. Yoshida E. Control of micellar size and critical micelle concentration for "nonamphiphilic" poly(vinyl phenol)-block-polystyrene diblock copolymers. Polym J 2003;35:965-71.
27. David A. Microemulsions. In: Kreuter J, editors. Colloidal Drug Delivery Systems, Drug and the Pharmaceutical Science, a Series of Textbooks and Monographs. New York: Marcel Dekker; 1994. p. 31-65.

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