Using Inverted Microemulsions for Transdermal Application of Folic Acid

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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 inside 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 $11.47 \pm 6.9 \times 10^{-4} \mu g/cm^2 \times h$ 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 $1.5 \pm 0.45 \text{h}$. Therefore, 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.

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MATERIALS, INSTRUMENTS AND METHODS

Materials

Folic acid was purchased from AZ Chem group (China). Sorbitan monolaurate (Span® 20) and Polyoxyethylene sorbitan monoolate (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. 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. 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.

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

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

1IPM: Isopropyl myristate, MEs: Microemulsions

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. 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 −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
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 accepter 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%.

\[
J_{ss} = \frac{Q/A}{t} = J_{ss} \times t
\]  
(1)

From Equation 1:

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

Where, \( K_p \): Permeability coefficient and \( C_v \): Vehicle concentration

From Equation 2:

\[
K_p = \frac{K_{sc} D_{sc}}{A \times \Delta x} = \frac{J_{ss}}{C_v}
\]  
(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
\( C_v \): The constant drug concentration in the donor solution

And \( \Delta x \): 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)](image-url)
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.

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

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

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 cm² were measured over 24 h and cumulative measured amount per cm² 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
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 fraction 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 ($\gamma$) to the amount of solute adsorbed at the interface ($\Gamma$).[25]

$$\tilde{\lambda} = \frac{a}{RT} \frac{d\gamma}{da}$$ (4)
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**


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