Design and Development of Microemulsion Drug Delivery System of Acyclovir for Improvement of Oral Bioavailability

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ABSTRACT

The main purpose of this work was to develop an oral microemulsion formulation for enhancing the bioavailability of acyclovir. A Labrafac-based microemulsion formulation with Labrasol as surfactant and Plurol Oleique as cosurfactant was developed for oral delivery of acyclovir. Phase behavior and solubilization capacity of the microemulsion system were characterized, and in vivo oral absorption of acyclovir from the microemulsion was investigated in rats. A single isotropic region, which was considered to be a bicontinuous microemulsion, was found in the pseudoternary phase diagrams developed at various Labrasol:Plurol Oleique:Labrafac ratios. With the increase of Labrasol concentration, the microemulsion region area and the amount of water and Labrafac solubilized into the microemulsion system increased; however, the increase of Plurol Oleique percentage produced opposite effects. The microemulsion system was also investigated in terms of other characteristics, such as interfacial tension, viscosity, pH, refractive index, diffusion, and bioavailability. Acyclovir, a poorly soluble drug, displayed high solubility in a microemulsion formulation using Labrafac (10%), Labrasol (32%), Plurol Oleique (8%), and water (50%). The in vitro intraduodenal diffusion and in vivo study revealed an increase of bioavailability (12.78 times) after oral administration of the microemulsion formulation as compared with the commercially available tablets.

KEYWORDS: Microemulsion, non-ionic surfactant, conductivity, interfacial tension, particle size.

INTRODUCTION

Acyclovir [9-(2-hydroxyethoxylmethyl) guanine], a synthetic purine nucleoside analog derived from guanine, is the most widely used antiviral agent. It is effective in the treatment of herpes simplex virus (HSV), mainly HSV-1 and HSV-2, and varicella-zoster virus.[1] The pharmacokinetic parameters of acyclovir following oral administration generally are highly variable. Peak plasma values have been shown to be 0.46 to 0.83 or 0.63 to 1.21 μg/L after a single oral dose of 200 or 400 mg, respectively,[2] and have been generally obtained 1.5 to 2.5 hours after oral administration.[2,3]

Acyclovir absorption in the gastrointestinal tract is slow, variable, and incomplete.[3] The bioavailability of acyclovir after oral administration ranges from 10% to 30%.[3] Approximately 80% of an oral dose is never absorbed and is excreted through the feces. The main excretory organ for acyclovir is the kidney. The plasma half-life of oral acyclovir on average is 3 hours in adults with normal renal function.[4]

In this study the use of a microemulsion to improve the extent of absorption and the overall bioavailability was investigated. This novel drug delivery system has been reported to improve the rate and extent of absorption of lipophilic drugs.[5-9] Microemulsions are homogeneous, transparent, thermodynamically stable dispersions of water and oil, stabilized by a surfactant, usually in combination with a cosurfactant (typically a short-chain alcohol). As pharmaceutical drug delivery systems, microemulsions have many advantages, including clarity, high stability, and ease of preparation.

In the present study, a microemulsion was prepared using the non-ionic Labrasol (as surfactant, hydrophile-lipophile balance [HLB] 16), Plurol Oleique (as cosurfactant, HLB 4), Labrafac, and water. Pseudoternary phase diagrams were constructed to find out the zone of microemulsion at different ratios of surfactant to cosurfactant (eg, 4:1 and 3:1). The effect of formulation variables on different physicochemical characteristics such as globule size, electroconductivity, and viscosity was studied. An ex vivo diffusion study was performed using rat duodenum, and the pharmacokinetics of the optimized microemulsion were evaluated by administering it orally to rats. The absolute and relative bioavailability was calculated after intravenous injection of a commercially available formulation (Acivir, Cipla, Mumbai, India) and oral administration of a commercial tablet (Aquivir, FDC, Mumbai India), respectively.

MATERIALS AND METHODS

Labrasol (caprylocaproyl macrogol-8-glycerides), Plurol Oleique (polyglyceryl-6-dioleate), and Labrafac (medium-chain triglyceride, C8-C10 fatty acids) (Gattefosse, Gennevilliers, France) were a gift of Colorcon Asia Ltd (Mumbai,

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India). Acyclovir was a gift from Alembic Ltd (Vadodara, India), and analytical-grade ammonium acetate (Sigma, Bangalore, India) and reagent-grade octane sulfonic acid sodium salt (Spectrum Lab, New Delhi, India) were used. High Performance Liquid Chromotography (HPLC)-grade methanol was purchased from SD Fine Chemicals (Maharashtra, India). All other chemicals used were of analytical reagent grade and used as received without further purification. Double-distilled water was used throughout the study.

Preparation of Microemulsion Formulation

Liquid microemulsions were prepared by dissolving Labrasol in Plurol Oleique. Acyclovir and Labrafac were then dissolved, followed by gentle mixing with distilled water. The monophasic formulations were formed spontaneously at room temperature. The final concentration of acyclovir in the microemulsions was 5%.

Construction of Phase Diagrams

Pseudoternary phase diagrams were constructed to examine the formation of oil in water microemulsions using 4 components: oil, surfactant, cosurfactant, and aqueous phase system. The 4-component system consisted of (1) a medium-chain fatty acid–based triglyceride (Labrafac); (2) a low-HLB (HLB = 4) surfactant (Plurol Oleique); (3) a high-HLB (HLB = 16) surfactant (Labrasol); and (4) double-distilled water (aqueous phase). Pseudoternary phase diagrams were constructed keeping the ratio of Labrasol and Plurol Oleique constant and varying the remaining 2 components. For convenience, the phase diagrams were constructed by drawing "water dilution lines" representing increasing water content and decreasing surfactant-cosurfactant levels.[10] The water was titrated along dilution lines drawn from the surfactant-cosurfactant apex (100% surfactant-cosurfactant) to the opposite oil side of the triangle. The line was arbitrarily denoted as the value of the line intersection with the oil scale (eg, 20:80, 30:70). If turbidity appeared followed by a phase separation, the samples were considered to be biphasic. If clear and transparent mixtures were visualized after stirring, the samples were considered monophasic. The samples were marked as points in the phase diagram. The area covered by these points was considered to be the microemulsion region of existence.

Physicochemical Evaluation

Particle Size Measurements

The droplet size of the emulsions was determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles[11]) using a Zetasizer 3000 (Malvern Instruments, Worcestershire, UK) able to measure sizes between 10 and 5000 nm. Light scattering was monitored at 25°C at a 90° angle, after external standardization with spherical polystyrene beads (63 nm).

Viscosity

The rheological property of the microemulsion was evaluated by a Brookfield LVDV 111 + CP viscometer (Stoughton, MA) at 30°C using a CPE 42 spindle at 5 rpm. Experiments were performed in triplicate for each sample, and results were presented as average ± standard deviation.

Electroconductivity Study

The electroconductivity of the resultant system was measured by an electroconductometer (CM 180 conductivity meter, Elco, Mumbai, India). For the conductivity measurements, the tested microemulsions were prepared with a 0.01N aqueous solution of sodium chloride instead of distilled water.

Refractive Index and Percent Transmittance

The refractive index of the system was measured by an Abbe refractometer (Bausch and Lomb Optical Company, Rochester, NY) by placing 1 drop of solution on the slide. The percent transmittance of the system was measured at 650 nm using a UV spectrophotometer (UV 1601, Shimadju, Japan) keeping distilled water as a blank.

In Vitro Intestinal Permeability Studies

The methods employed were modified from experimental procedures well described in the literature.[12] Male Sprague-Dawley rats (250-300 g) were killed by overdose with pentobarbitone administered by intravenous injection. To check the intraduodenal permeability, the duodenal part of the small intestine was isolated and taken for the in vitro diffusion study. Then this tissue was thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. The microemulsion sample was diluted with 1 mL of distilled water (outside mixing for 1 minute by vortex mixer), and for the tablet sample a suspension of tablet was made in distilled water. The resultant sample (2 mg/mL) was injected into the lumen of the duodenum using a syringe, and the 2 sides of the intestine were tightly closed. Then the tissue was placed in a chamber of organ bath with continuous aeration and a constant temperature of 37°C. The receiver compartment was filled with 30 mL of phosphate-buffered saline (pH 5.5). The absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 252 nm, keeping the respective blank. The percent diffusion of drug was calculated against time and plotted on a graph.

In Vivo Absorption Study

Absorption studies were performed in male albino Sprague-Dawley rats weighing 280 to 350 g. All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee of MS University of Baroda and are in accordance with the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. The animals were fasted overnight prior to the experiment but had free access to water. The microemulsion was administered by oral snode in an equivalent dose of 19 mg/kg of acyclovir. The tablet suspension was administered in the same manner to the second group of rats. Intravenous administration of the acyclovir injection (same dose) was also given to another group of rats (3 rats). The blood samples (approximately 300-400 µL) were collected from the retro-orbital vein using a heparinized needle (18-20 size) at 0, 5, 10, 30, 45, 60, 120, and 240 minutes after intravenous administration and 0, 0.5, 1, 2, 3, 4, 6, 12, and 24 hours after oral administration. The blood samples were collected into a heparinized microcentrifuge tube. Then the samples were subjected to centrifugation on a laboratory centrifuge (Sigma, 3K30) at 10 000 rpm for 10 minutes at 0°C, and supernatant plasma was collected into another microcentrifuge tube and kept at –20°C until analysis.

HPLC Analysis of Plasma Sample

The concentration of acyclovir in plasma samples was determined by HPLC analysis. The HPLC system consisted of Hewlett-Packard (Agilent) 1100 series components, including a quaternary pump, auto sampler, and variable-wavelength UV detector (Palo Alto, CA). The acyclovir was detected at 252 nm. Chromatographic separations were achieved using an Inertsil ODS-3V column (250 × 4.6 mm, 5 µm) (GL Science, Tokyo, Japan). The mobile phase used for the plasma sample was 20mM ammonium acetate with 5mM octane sulfonic acid sodium salt in water (pH adjusted to 3 by acetic acid)-methanol (98:2, vol/vol). Filtration of the buffer was done using 0.2µm nylon 6.6 membrane filters, and it was degassed by sonication.

Exactly 200 µL of the thawed plasma samples was mixed with 100 µL of water by vortexing for 1 minute using a Vibromixer (SPINIX, Mumbai, India). To this was added 20 µL of 35% perchloric acid, and the solution was mixed for 1 minute for protein precipitation. Then this mixture was centrifuged at 10 000 rpm for 10 minutes using a Biofuge Pico Micro centrifuge (Heraeus Instruments, Hanau, Germany). After centrifugation, 50 µL of supernatant solution was injected into the HPLC system. The linearity of the method was found suitable in the range of 0.05 to 10 µg/mL (R^2 = 0.9999).

Pharmacokinetic Data Analysis

For oral and intravenous administration, the area under the drug concentration-time curve from 0 to 24 hours (AUC) was calculated using the 2-compartment mode of WinNonlin Software 4.11[13] for pharmacokinetic analysis.

Statistical Analysis

The data from different formulations were compared for statistical significance by 1-way analysis of variance (ANOVA). Differences were considered to be statistically significant when $P < .01$.

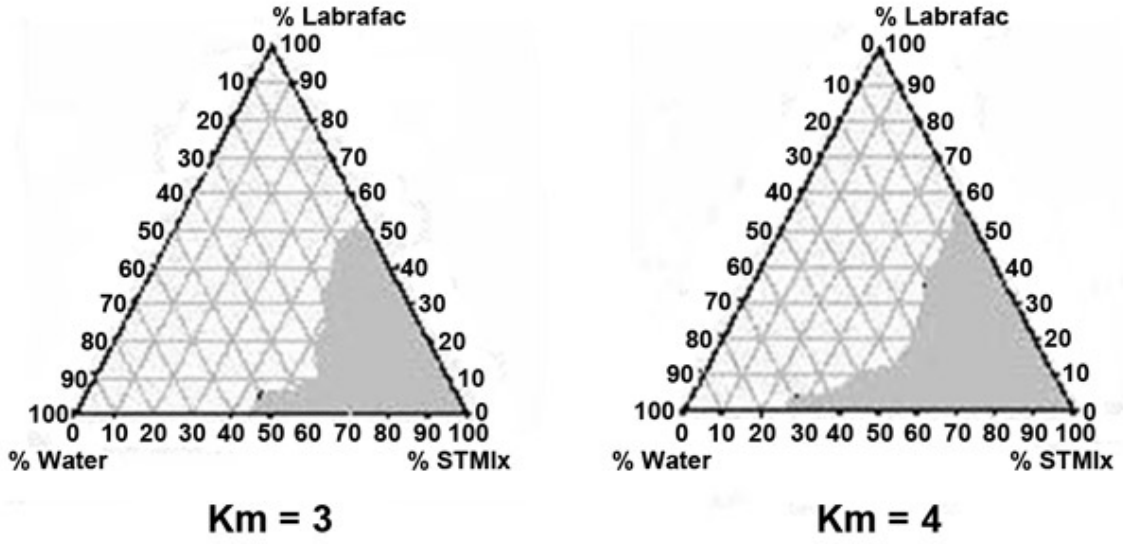

Figure 1. Pseudoternary phase diagram of Labrasol, Plurol Oleique, Labrafac, and water. (a) Km = 3, (b) Km = 4. Km indicates surfactant to cosurfactant ratio; STMix indicates surfactant + cosurfactant.

Table 1. Physicochemical Parameters of the Developed Microemulsion

Parameter	Value
Particle size (nm)	40.2 ± 2.3
Particle size after dilution by 100 times with water (nm)	551 ± 24
Electroconductivity (μΩ)	254 ± 3
Viscosity (cP)	27.5 ± 1
Refractive index	1.34
Percent transmittance at 650 nm	99.4 ± 0.4

Results and Discussion

Phase Diagram Study

A pseudoternary phase diagram of the investigated quaternary system water/Labrasol/Plurol Oleique/Labrafac is presented in Figure 1. Formation of microemulsion systems (the shaded area) was observed at room temperature. Phase behavior investigations of this system demonstrated the suitable approach to determining the water phase, oil phase, surfactant concentration, and cosurfactant concentration with which the transparent, 1-phase low-viscous microemulsion system was formed.

The phase study revealed that the maximum proportion of oil was incorporated in microemulsion systems when the surfactant-to-cosurfactant ratio (km) was 4:1. From a formulation viewpoint, the increased oil content in microemulsions may provide a greater opportunity for the solubilization of acyclovir. Moreover, when the composition (% wt/wt) of surfactant mixture (S_{mix}) in a microemulsion preparation was <40%, the formulation was less viscous. The optimum formulation of microemulsion contained Labrafac (10%), Labrasol (32%), Plurol Oleique (8%), and water (50%).

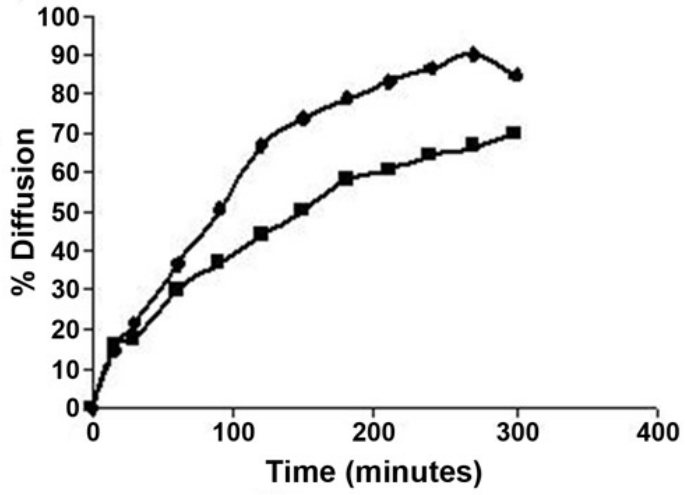

Figure 2. Comparative in vitro diffusion profile of acyclovir through rat duodenum (–♦–) for microemulsion (ME) and (–■–) for tablet.

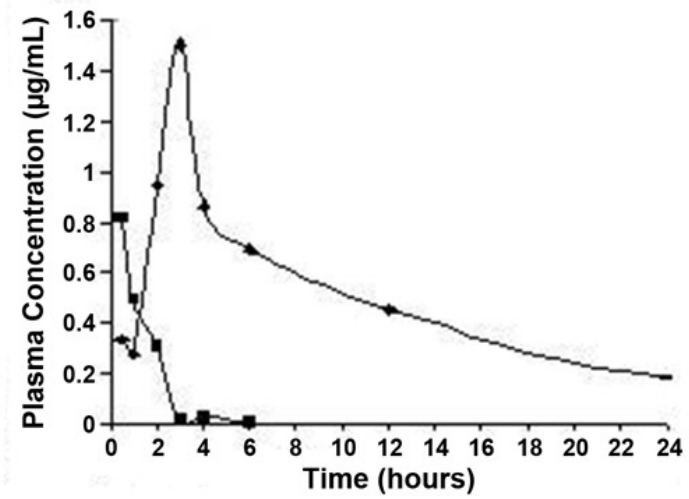

Figure 3. Comparative plasma concentration of acyclovir after oral administration of (–♦–) ME and (–■–) tablet. ME indicates microemulsion.

Physicochemical Characterization of Microemulsion

The physicochemical characteristics of the developed microemulsion appear in Table 1. It was clear from the physicochemical data that the developed system had low viscosity (~27.46 cP). The investigated microemulsion system containing the non-ionic surfactant mixture, oil, and water showed electroconductive behavior in spite of its non-ionic nature. From the viscosity and electroconductive study it can be concluded that the system is of the o/w type.[14] The refractive index of the developed system was similar to the refractive index of water (1.333). In addition, the developed system showed percent transmittance >99%. The refractive index and percent transmittance data prove the transparency of the system. The nanometric size range of the particle was retained even after 100 times dilution with water, which proves the system's compatibility with excess water. Similar behavior was observed in a Labrasol system without any drug.[14]

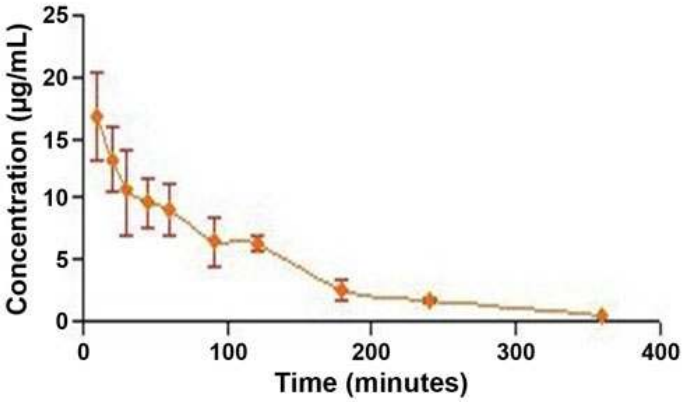

Figure 4. Plasma concentration of acyclovir after intravenous administration.

Table 2. Summary of Pharmacokinetic Data of Acyclovir in Rats Following Intravenous and Oral Administration of 19 mg/kg of Acyclovir*

Parameter	Intravenous Administration	Tablet Oral Administration	ME Oral Administration
Co (μg/mL)	36.35	—	—
T_{max} (hr)	—	0.5	3.0
C_{max} (μg/mL)	—	0.813	1.508
$T_{1/2}$ (hr)	0.873	0.542	9.070
$AUC_{0\text{-}t}$ (μg/min/mL)	49.29	1.111	12.02
$AUC_{0\text{-}\acute{a}}$ (μg/min/mL)	51.4	1.120	14.32
Kel	0.0131	0.0160	0.0124
Absolute bioavailability† (%)	—	2.18	27.8
Relative bioavailability‡ (%)	—	—	12.8

*ME indicates microemulsion; AUC, indicates area under the curve, and; IV, intravenous.

†Absolute bioavailability = $(AUC_{oral}/AUC_{IV}) \times (dose_{IV}/dose_{oral})$.

‡Relative bioavailability = $(AUC_{ME}/AUC_{tab}) \times (dose_{Tab}/dose_{ME})$. Mean ± standard deviation, n = 3-4.

In Vitro Intestinal Permeability Study

In vitro intestinal permeability data are shown in Figure 2. The drug diffused at a faster rate from the microemulsion system than from the tablet dosage form. The total percentage diffusion was much higher for the microemulsion system than for the tablet dosage form. After 5 hours of diffusion, 85% of the drug was diffused from the microemulsion system, as compared with 69% diffused from the tablets.

Pharmacokinetic Data

The plasma concentration vs time profile after oral administration is shown in Figure 3; the profile after intravenous administration appears in Figure 4. The pharmacokinetic parameters are in Table 2. The C_{max} of tablet formulation was 0.813 μg/mL after 30 minutes, whereas it was 1.508 μg/mL for the microemulsion formulation after 3 hours. This may have been due to the slow diffusion of acyclovir from the dispersed oil globules to the continuous medium. But the release of drug was greater and more sustained from the microemulsion formulation than from tablets.

The enhanced absorption may be explained in terms of (1) the huge specific surface area of the microemulsion droplets (mean droplet size ~40 nm), (2) improved permeation of the acyclovir because of the presence of surfactant, which reduces the interfacial tension to nearly 0; and (3) the stability of the microemulsion in the gastrointestinal tract. A statistically significant difference ($P < .01$) between the 2 formulations was found from the ANOVA analysis.

CONCLUSION

The study demonstrates that the microemulsion formulation can be employed to improve the bioavailability of a poorly absorbed drug. The ratio of Labrasol:Plurol Oleique:Labrafac played a major role in formulating the microemulsion. The optimum microemulsion formulation contained Labrafac (10%), Labrasol (32%), Plurol Oleique (8%), and water (50%), which was a transparent and less viscous system. After oral administration in rats, the microemulsion showed an absolute bioavailability of 27.83%, which is 12.78 times higher than that of commercially available tablets (Aquivir).

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