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Development Studies On Microemulsion Gels Containing Herbal Excipients as Permeation Enhancers

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ABSTRACT

Objective: Acyclovir is an effective antiviral medication used in the management of Herpes Simplex Virus (HSV) infections. Although acyclovir sodium exhibits high solubility, it has low permeability, resulting in suboptimal bioavailability, necessitating elevated dosages, which can lead to increased side effects. The objective of this study was to formulate an oral microemulsion gel utilising a herbal excipient to enhance the permeability and bioavailability of acyclovir, thereby minimising the required doses and associated adverse effects.

Methods: Microemulsions were developed by systematically titrating different oil-to-surfactant ratios in an aqueous phase, as illustrated in a pseudo-ternary phase diagram. The evaluation included parameters, such as clarity, dilution shock, in vitro drug release, and ileal permeability. This method offers advantages such as reduced processing time, minimal exposure to high temperatures, and effective drug-loading efficiency.

Findings: The stable microemulsion gel composed of Captex 200, Tween 80, ethanol, water, propylene glycol, 100% Aloe vera, and 2% Carbopol demonstrated a fourfold enhancement in ileal permeability when compared to the acyclovir sodium solution.

Novelty: Aloe vera gel improves the permeation of microemulsions, thereby enhancing the intestinal permeability and bioavailability of BCS class III pharmaceuticals. This formulation has the potential to be administered through soft gel capsules.

Keywords: Acyclovir sodium; Aloe vera; BCS class III drugs; Permiability 200; Microemulsion gel; Permeability

INTRODUCTION

Microemulsions (MEs) are stable, fluid, transparent, or translucent homogenous dispersions with a quaternary composition of oil, aqueous phase, surfactant, and cosurfactant, forming a single optically isotropic dispersion with droplet diameters typically 10–100 nm[1–5]. Microemulsions (MEs) have been extensively used to improve skin penetration of compounds with low water solubility and high lipophilicity[1]. A surfactant/co-surfactant mixture was used to emulsify two immiscible phases, the oil and aqueous phases, resulting in a transparent and thermodynamically stable formulation[6]. Acyclovir [2-amino-9-(2-hydroxyethoxymethyl)-3H-purin-6-one], a synthetic purine nucleoside analog derived from guanine, is a BCS class III drug used to treat herpes simplex virus (HSV-1, HSV-2) and varicella-zoster virus[7]. Its pharmacokinetics after oral administration are highly variable, with peak plasma values of 0.46 to 0.83 μg/L for a 200 mg dose or 0.63 to 1.21 μg/L for a 400 mg dose occurring 1.5 to 2.5 hours post-dose. Acyclovir is administered orally, via intravenous infusion, and topically[8]. This study developed an oral formulation of acyclovir using microemulsions and aloe vera gel as permeation enhancers, examining the impact of surfactants, cosurfactants, and a lipoidal system on the ileal permeability of acyclovir.

METHODOLOGY

Materials

Acyclovir in the form of sodium salt was generously gifted by Strides Arco Labs in India. Several oils were utilised, including Capmul MCM (Glyceryl Mono-Dicaprylate), Captex 200 (mixed diesters of caprylic/capric acids on propylene glycol) and Capmul PG-8 (Propylene glycol caprylate) were procured from ABITEC in the USA. The

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surfactants incorporated in the formulation were Span 80 and Span 20 from Sigma-Aldrich in the USA, Tween 80 from Merck in India, Labrasol (polyglycolized glycerides) from Gattefosse in France, and Acconon CC-6 (ethoxylated caprylic and capric glycerol esters) from ABITEC in the USA. Cosurfactants included absolute ethanol sourced from Merck in India and Plurol Oleque (Polyglyceryl-6-dioleate) from Gattefosse in France. Aloe vera, used as a permeation enhancer, was gifted by Sagar Pharmaceuticals in India. Additionally, the polymer carbopol 974-P was sourced from Strides Arco Labs in India.

The study utilised a UV/VIS spectrophotometer, electronic balance, vortex shaker, micropipettes, and pH meter. Particle size and zeta potential analysers were used for particle analysis, and an IR Spectrometer was used for infrared spectroscopy. The water was semi-quartz distilled, and all chemicals and reagents were of A.R. grade, commercially procured, and used.

Physicochemical Study on the Drug

Melting point determination

Thiel's tube method of melting point determination in liquid paraffin was used in the present study[9].

UV spectrum

UV spectrum scanning for the pure drug was conducted within the range of 200-300 nm. In the dilution medium of NaOH (0.1N), the maximum wavelength (λ_{max}) was observed at 265 nm. When using a dilution medium at 6.8 pH Phosphate buffer, λ_{max} was identified at 251.5 nm. Additionally, in a dilution medium of the Tyrode solution, the λ_{max} was determined to be 252.5 nm. Three standard calibration curves for acyclovir were plotted using 0.1N NaoH, phosphate buffer (pH 6.8), and Tyrode solution as the medium[9].

Formulation

Selection of surfactant and co surfactant

A series of Surfactants with different HLB values have been screened such as Span 20, Span 80, Tween 80, Labrasol, Acconon CC-6 and Co- Surfactant like Ethanol and Plurol Oleque. Drug solubility studies were performed using different surfactants and co-surfactants. We found that Tween 80, in combination with a co-surfactant, ethanol, was the best for formulating a microemulsion.

Construction of Phase diagram

A pseudo-ternary phase diagram was created to study the formation of microemulsions using four components: oil surfactant, co-surfactant, and aqueous phase system. The system consisted of oil as Captex 200, surfactants such as Tween 80, co-surfactant ethanol, and double distilled water as the aqueous phase. A phase diagram was created by drawing water dilution lines representing increasing water content and decreasing surfactant and co-surfactant levels. Water was titrated along the dilution lines from the surfactant apex to the opposite oil side of the triangle. If turbidity was followed by phase separation, the mixture was considered to be a biphasic dispersion. Clear and transparent mixtures were considered microemulsions. The transition point samples were marked as points in the phase diagram, and the area covered by these points was considered as the microemulsion region of existence[10,11].

Formulation Development

After the development of the phase diagram, different formulations were selected by keeping the total quantity of the formulation constant at 100% and varying all the components of the system. Each formulation was loaded with Acyclovir Na (5.1 mg/ml). All formulations were evaluated for different parameters such as clarity and dilution shock. The optimised formulation (which was selected from the data obtained from the evaluation studies) was further evaluated for in vitro release and intestinal permeability studies.

Effect of Aloe vera on Optimized Formulation

The optimised formulation was mixed with different concentrations of aloe vera (25, 35, 50, and 100% w/w). The formulations were then evaluated for in vitro release and intestinal permeability.

Effect of Aloe vera and Carbopol on Optimized Formulation

A microemulsion gel of the optimised formulation was formed with 2% carbopol 974-P which was further mixed with different concentrations of aloe vera (25%, 35%, 50%, and 100%). The formulations were then evaluated for in vitro release and intestinal permeability.

Evaluation Studies

Clarity

The clarity of all formulations was determined by measuring their absorbance and % transmittance at 650 and 400 nm, respectively[8].

Dilution Shock test

Each formulation was diluted 10 and 25 times, and their absorbance and % transmittance were noted at 650 nm and 400 nm, respectively[12].

Particle size analysis

The particle sizes of the microemulsions were analysed using a Malvern particle size analyser.

Zeta potential

The zeta potential of the microemulsions was measured using a Malvern Zetasizer.

In vitro drug release study

The release study used a dialysis membrane (HIMEDIA 70 LA 393-10MT) activated with 5% EDTA solution and release medium. A phosphate buffer (pH 6.8) was used as the release medium. The membrane was suspended in a beaker containing 250 ml of buffer solution, stirred on a magnetic stirrer, and poured into a dialysis membrane tube. Samples were removed and diluted with phosphate buffer solution at intervals of 5, 10, 15, 30, 45, and 60 min and diluted to 10 ml with phosphate buffer solution. The absorbance of each sample was noted at 251.5 nm [13]

In vitro intestinal permeability study

The rats were euthanised with a chloroform overdose. The ileum from the small intestine was cut and removed and then placed in a Tyrode solution at 37 °C with aeration. The tissue was washed with Tyrode's solution to remove the extraneous luminal matter. A sample of 1.5 mg/ml was transferred to the tissue, tied, and placed in a beaker containing 50 ml of tyrode. The samples were removed at different intervals and diluted to 10 ml. The absorbance of these solutions was measured using a UV/VIS spectrophotometre at a wavelength of 252.5 nm, while the tyrode was kept blank. The same experiment was conducted for the blank to check for any absorbance in the tissue or tyrode solution [14].

RESULTS AND DISCUSSION

Most drugs are structurally optimised during drug discovery studies to increase their solubility and efficiency of bio membrane permeation to reach their target receptors. While an increase in lipophilicity helps in better absorption, their delivery to the aqueous physiological milieu becomes difficult. On the other hand, some drugs have good water solubility but lack sufficient lipophilicity to effectively penetrate the lipoidal tissues, and absorption becomes rate-limiting for such candidates. Microemulsions, owing to their unique structure and dynamics [15], afford efficient delivery of both lipophilic and hydrophilic drugs in aqueous and lipoidal environments. Owing to its absorption-enhancing effects, Aloe vera gel has been reported to effectively deliver poorly absorbable drugs through the oral route of drug administration [16]. This study investigated the potential of a synergy of microemulsion and aloe vera gel to improve the absorption of drugs with low solubility or permeability. Acyclovir, a synthetic purine nucleoside with low permeability, is a model drug that can be improved by formulating it in a microemulsion system and incorporating aloe vera gel. The formulation development process involves pre-formulation studies, analytical investigations, method selection, standardisation, validation, and preliminary trials. The study also focused on selecting suitable oils, surfactants, and cosurfactants for oral administration, as well as studying their impact on the physicochemical and pharmaceutical properties of dosage forms.

The oral microemulsion of acyclovir underwent pre-formulation studies, including melting point and UV spectra analysis, to ensure it met Pharmacopoeial standards for melting point and wavelength of maximum absorption (λmax). After ensuring the purity of the drug and verifying its λmax in an appropriate solvent, a standard calibration curve was prepared, showing a linear relationship from 2 μg/ml to 20 μg/ml at 265 nm in 0.1N NaOH. Further analysis at 251.5 nm in phosphate buffer (pH 6.8 and 252.5 nm in Tyrode solution also showed linearity in the same concentration range. The study screened various surfactants and cosurfactants for drug solubility, with Tween 80 and ethanol being the most effective for formulating a microemulsion based on their HLB values and their combination with ethanol.

A pseudo-ternary phase diagram technique was used for microemulsion formulation. The oil was mixed with surfactants and cosurfactants and then titrated with water to form a turbid emulsion. The phase diagram was plotted as a triangle, with the weights of the oil, water, and surfactant mixture at different ratios. To optimise the formulation, specific points in the phase diagram were selected from the microemulsion zone. Each formulation was evaluated for clarity, dilution shock, in vitro release, and in vitro intestinal permeability. The results for each parameter are then discussed.

All three formulations exhibited clear, transparent, and pale yellow colours. Clarity is represented in terms of % transmission, with absorbance and % transmission noted at 650 nm and 400 nm, respectively. The best results were reported at 650 nm, with transmission exceeding 80% for all formulations. Visually, all formulations showed clear microemulsion formation owing to the minimal adjustment time required for surfactant and cosurfactant molecules to orient at the interface and achieve the necessary curvature for thermodynamic stability. The formulations were diluted with distilled water to 25X and 50X for dilution stock studies and their absorbance and % transmission was measured at 650 nm and 400 nm, respectively. All formulations maintained good clarity after dilution, with formulation 2 maintaining the best clarity even after 25X and 50X dilution (evidenced by a high %T near 90% and absorbance < 0.3). Additionally, formulation 2 had the best drug-loading capacity (5.1 mg) compared to the other formulations. The advantage of the system lies in its ability to maintain clarity and resist haziness after reaching the GIT, maintaining particle size in the microemulsion range, and increasing solubility and bioavailability due to the higher surface area of the nanodroplets (Table 1).

Table 1: Preformulation study on acyclovir sodium

Melting Point			
Reported		Observed	
256.5-257 °C		256 °C	
Drug solubility studies			
Sr. No	**Category**	**Components**	**Drug loading capacity (mg)**
1	Surfactant	Labrasol	1.7
		Acconon CC-6	2.1
		Span 80	0.9
		Span 20	1.1
		Tween 80	3.6
2	Co-Surfactant	Absolute Ethanol	2.8
		Plurol Oleque	1.6
		PEG-400	1.1
		Glycerol	0.9
		Sorbitol	0.7
3	Aqueous Solvent	Water	97.5
		Propylene glycol	10.7
		Benzyl alcohol	2.3
		Isopropyl alcohol	1.2
4	Oil	Capmul MCM	0.7
		Captex 200	1.4
		CapmulPG-8	0.9

Final Formulation Development Phase				
Formulation code	**% oil**	**% S+C**	**% Aqueous Phase**	**Drug loading (mg)**
FM 1	2.5	47.5	50	3.8
FM 2	3.5	46.5	50	5.1
FM 3	4.5	45.5	50	2.9

Clarity		
Formulation code	**% Transmission at 650 nm**	**% Transmission at 400 nm**
FM 1	79.6	73.2
FM 2	89.6	81.5
FM 3	83.5	75.7

Dilution shock test				
Formulation Code	**Dilution 1:25**		**Dilution 1:50**	
	% Transmission			
	AT 650nm	**AT 400nm**	**AT 650nm**	**AT 400nm**
FM 1	91.7	86.4	94.3	90.2
FM 2	93.5	90.7	96.7	92.3
FM 3	92.9	88.3	96.1	92.5
Formulation code	**Dilution 1:25**		**Dilution 1:50**	
	Absorbance			
	AT 650 nm	**AT 400 nm**	**AT 650 nm**	**AT 400 nm**
FM 1	0.038	0.064	0.025	0.044
FM 2	0.029	0.041	0.022	0.038
FM 3	0.031	0.054	0.023	0.038

The in vitro drug release study revealed that the drug release after 1 h for the optimised microemulsion formulation (F2) was 55% ±0.952, compared to 91% ±1.938 for the pure drug (Table 2).

This indicates a slowdown in drug release from the microemulsion, likely due to reduced diffusion of the drug's sodium salt through the lamellar surfactant/cosurfactant layers, which increases the microenvironment viscosity and may suggest migration of the drug into the oily phase or entrapment in the surfactant-cosurfactant layers. When different concentrations of aloe vera (ranging from 25% to 100%) were mixed with the optimised formulation, drug release increased with higher aloe vera concentrations: 62% ±1.42 for 25% aloe vera, 69% ±1.67 for 35% aloe vera, 73% ±6.37 for 50% aloe vera, and 80% ±9.66 for 100% aloe vera (Table 2). This increase is likely due to the hydrophilic Aloe vera causing relaxation of the lamellar structure of the surfactant-cosurfactant layers, allowing more drug to diffuse out. However, in the presence of 2% carbopol and varying concentrations of aloe vera, there was a decrease in drug release: 65% ±14.03 with 25% aloe vera, 61% ±11.02 with 35% aloe vera, 52% ±7.83 with 50% aloe vera, and 45% ±6.74 with 100% aloe vera (Table 3).

This reduction was attributed to the higher viscosity of 2% carbopol, which lowered the diffusion coefficient. After 1 h of permeation, most of the microemulsion formulations showed enhanced permeation compared to the drug solution. Permeation after 1 h from the optimised formulation was 31% ±0.91, as opposed to only 18% ±1.09 for the pure drug (Table 2). This is in line with the BCS classification of the drug into class III (high solubility, 91% drug release after 1 h; low permeability). Different concentrations of aloe vera gel, ranging from 25% to 100%, were mixed with the optimised formulation. Drug permeation after 1 h from 25% aloe vera was 48% ±1.27, from 35% aloe vera was 55% ±1.31 (Table 2), from 50% aloe vera was 59% ±1.05, and from 100% aloe vera was 76% ± 4.43 (Table 3). Aloe vera enhances drug permeation by reversibly opening tight junctions, allowing better drug penetration. As aloe vera concentration increased, drug permeation also increased. However, its impact on ileal membrane structure remains unexplored. Drug permeation from microemulsions with 2% carbopol and different aloe vera concentrations showed a 29% ±1.89 rate at the end of 1 h, with 35% aloe vera and 2% carbopol 35% ±1.13, 50% aloe vera and 2% carbopol 44% ±1.11, and 100% aloe vera and 2% carbopol 58% ±9.82 (Table 3). Carbopol caused a 20% reduction in permeability, affecting drug release and potentially reducing drug availability owing to increased viscosity.

CONCLUSION

Acyclovir was formulated into a microemulsion gel system, which was then transformed into a microemulsion gel with

Table 2: In vitro drug release and permeation

In vitro drug release for pure drug		
Time (min)	**% CDR Average (Pure drug in distilled water at 5.1 mg/mL)**	**% CDR Average (Optimised formulation (f2) in Phosphate buffer)**
5 min	20.366 ± 6.215	12.43 ± 1.030
10 min	30.516 ± 2.823	14.29 ± 1.411
15 min	39.34 ± 6.161	18.75 ± 0.610
30 min	54.43 ± 5.837	33.3 ± 1.170
45 min	72.653 ± 6.082	44.4 ± 1.056
60 min	91.416 ± 1.938	55.36 ± 0.952
In vitro drug permeation for pure drug		
Time in min	**% CDR Average (Pure drug in distilled water at 5.1 mg/mL)**	**% CDRAverage (Optimized formulation f2-in tyrode solution)**
5 min	6.77 ± 0.412	8.70 ± 0.496
10 min	8.6 ± 0.512	13.42 ± 1.276
15 min	12.33 ± 1.597	16.98 ± 0.25
30 min	13.52 ± 1.266	20.66 ± 0.502
45 min	15.46 ± 0.705	24.65 ± 1.524
60 min	18.26 ± 1.097	31.3 ± 0.901

In vitro drug release and permeation for formulation		
Time (min)	**Drug release % CDR (Formulation containing drug solution in distilled water at 5.1 mg/ml and 100% aloe vera)**	**Drug permeation% CDR (Formulation containing drug solution in distilled water at5.1 mg/ml and 100% aloe vera)**
5	43.6	11.2
10	54	16
15	66.4	20.8
30	78.8	30.4
45	87.2	± 38
60	95.6	9± 40.4

Release study of optimized formulation				
Time in min	**% CDR Average (25% Aloe vera)**	**% CDR Average (35% Aloe vera)**	**% CDR Average (50% Aloe vera)**	**% CDR Average (100% Aloe vera)**
5 min	13.87 ± 1.330	20.57 ± 1.866	21.93 ± 0.230	20.67 ± 3.422
10 min	19.82 ±0.346	26.17 ± 0.739	28.1 ± 1.11	27.84 ± 3.424
15 min	28.63 ± 0.461	31.70 ± 0.950	38.82 ± 3.011	41.07 ± 4.411
30 min	42.78 ± 0.956	47.68 ± 0.493	53.99 ± 3.677	55.79 ± 1.200
45 min	52.19 ± 0.661	60.09 ± 2.201	66.71 ± 4.232	73.29 ± 9.031y
60 min	61.86 ± 1.428	69.07 ± 1.679	73.56 ± 6.372	79.78 ± 9.664

Carbopol and Aloe vera gel. The microemulsion showed improved drug release, with Aloe vera gel enhancing permeation. The study concluded that Aloe vera gel can significantly improve the intestinal permeability and bioavailability of BCS class III drugs through a microemulsion platform. Future studies could include whole-animal pharmacokinetic studies and human clinical trials.

Table 3: Permeation, release and permeability study of optimized formulation

Permeation study of optimized formulation				
Time in min	**%CDR Average (25% Alora)**	**%CDR Average (35% Alora)**	**%CDR Average (50% Alora)**	**%CDRAverage (100% Alora)**
5	11.38 ± 0.792	12.16 ± 2.772	14.66 ± 2.552	16.36 ± 2.502
10	14.41 ± 0.547	14.97 ± 1.390	17.59 ± 0.760	21.76 ± 2.244
15	17.38 ± 0.665	19.76 ± 0.465	22.17 ± 0.918	26.11 ± 1.654
30	24.94 ± 1.530	27.54 ± 1.640	28.43 ± 1.029	30.17 ± 1.031
45	37.80 ± 1.096	39.28 ± 0.906	42.08 ± 1.974	48.68 ± 1.244
60	48.67 ± 1.270	54.80 ± 1.315	59.25 ± 1.057	76.39 ± 4.432
Release study of optimized formulation with 2% carbopol and 25% aloe ver				
Time in min	**%CDR Average (2% Carbopol and 25% Aloe vera)**	**%CDR Average (2% Carbopol and 35% Aloe vera)**	**%CDR Average (2% Carbopol and 50% Aloe vera)**	**%CDRAverage (2% Carbopol and 100% Aloe vera)**
5 min	7.20 ± 0.987	4.75 ± 0.447	6.38 ± 0.626	4.94 ± 1.939
10 min	16.60 ± 1.612	12.71 ± 0.86	10.86 ± 0.419	8.23 ± 2.574
15 min	22.67 ± 3.026	22.29 ± 1.124	20.45 ± 0.929	19.72 ± 5.015
30 min	33.7 ± 2.683	29.79 ± 1.291	27.73 ± 1.136	26.22 ± 5.675
45 min	49.50 ± 2.058	43.81 ± 1.510	39.97 ± 3.310	38.36 ± 7.379
60 min	65.83 ± 14.035	61.47 ± 11.021	52.24 ± 7.833	45.21 ± 6.740
Permeability study of optimized formulation				
Time in min	**%CDR Average (2% Carbopol and 25% Aloe vera)**	**%CDR Average (2% Carbopol and 35% Aloe vera)**	**%CDR Average (2% Carbopol and 50% Aloe vera)**	**%CDR Average (2% Carbopol and 100% Aloe vera)**
5 min	1.66 ± 0.650	3.05 ± 0.783	6.11 ± 0.57	11.55 ± 2.785
10 min	4.09 ± 0.525	5.43 ± 0.981	8.75 ± 0.441	18.82 ± 4.421
15 min	6.84 ± 1.086	7.82 ± 0.842	12.29 ± 1.006	24.03 ± 5.069
30 min	13.69 ± 1.615	17.42 ± 1.011	22.49 ± 0.903	33.95 ± 6.262
45 min	20.08 ± 1.551	24.50 ± 0.953	31.34 ± 0.794	43.92 ± 8.129
60 min	28.89 ± 1.891	35.43 ± 1.131	44.73 ± 1.119	57.87 ± 9.825

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