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Research Article

Formulation And Development of Novel Microemulsion Of Acyclovir

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ABSTRACT

The aim of this study was to enhance the permeability of acyclovir (ACV) by formulating a water-in-oil microemulsion. Permeability plays a crucial role in the oral absorption and bioavailability of drugs. The microemulsion (ME) was characterized using droplet size analysis, dilution test, zeta potential measurement, in vitro drug permeation study, and in vivo study. The optimized formulation, which demonstrated the highest ACV incorporation (81.57 mg/ml), comprised 13.3% Polysorbate 80, 13.3% ethanol, 6.6% ginger oil, and 66% water. The droplet size analysis indicated optimal results, and other parameters met the desired criteria. These findings underscore the ME's capability to enhance ACV permeability, highlighting its potential to enhance the effectiveness of orally administered ACV pharmaceutical formulations.

INTRODUCTION

Micro emulsions are thermodynamically stable dispersions of oil and water stabilized by a surfactant and, in many cases, also a co surfactant. They have characteristic properties such as ultralow interfacial tension, large interfacial area and capacity to solubilise both aqueous and oil-soluble compounds. Hence hydrophobic as well as hydrophilic groups can be incorporated into microemulsion drug delivery systems. Present study aims at establishing better dosage forms of acyclovir which would be safe, efficacious, stable and patient compliant.

Idea of microemulsions was first presented by Hoar and Schulman amid 1940s. It is an arrangement of water, oil and surfactant, which is optically isotropic and thermodynamically stable fluid that has low consistency or interfacial film comprising of surfactant/co-surfactant [1]. It is the vehicle for enhancing drug delivery, optimization of dose and bioavailability of drugs [2]. It is well-established that medium-chain unsaturated fats impact tight intersections of epithelial cells, and long-chain unsaturated fats allow lipoprotein amalgamation and consequential lymphatic absorption. Lately, various investigations have suggested that microemulsion [o/w or w/o] can promote the bioavailability of medications using topical routes.

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A drug delivery system in which a drug gets released in a controlled and desired manner to provide the required therapeutic effect is a successful drug delivery system. The drug delivery system should also provide the drug at the desired site at the desired time which is ubiquitous and spatial delivery. The dosage form should be stable, rational, robust, safe and patient-compliant [2,3].

Key interest in the microsponge advancement originates from the fact that the conventional drug delivery system had certain disadvantages. Microsponge systems can incorporate high quantities of the drug into themselves and hence provide the desired action for a prolonged period. They can eliminate the inconvenience caused by the repeated administration of the drug in conventional forms.

Herpes genitals a sexually transmitted disease caused by herpes simplex virus (HSV), DNA virus that has two serotypes: HSV-1 and HSV-2. HSV-1 is responsible for virtually all cases of oral herpes and for approximately 50% of the first episode of genital infections [4,5].

Herpes Statistics: Across the nation, one out of five of the populaces, have HSV-1 or 2. Upwards of 1 of every 4 individuals under the age of 25 in the United States have HSV 1 or 2. HSV-2 disease is more common in ladies than in men. Measurements show that the number of Americans with genital herpes contamination has expanded by 30%. The biggest increment is happening in youthful white teenagers. HSV-2 contamination is presently five times more typical in 12 to 19-year-old whites and it's twice as regular in youthful grown-ups ages 20 to 29. By the time individuals achieve their 40s, just about 90% of them have HSV 1 or 2. That implies everybody looks for herpes sooner or up some other time, regardless of whether they don't know they have it.

If you have herpes, then it turns out to be simple for you to be contaminated with HIV infection. Herpes can prompt numerous other hurtful maladies. You don't need to stress, however; herpes can be dealt with effectively. Science has thought of approaches to back off and annihilate herpes infection. In any case, because of its profoundly infective state, it can demonstrate difficult and unsafe for patients to engage in sexual relations. Furthermore, repeat injuries can make mental misery persistent. In any case, there are two perilous complexities associated with herpes disease. To start with, there is the plausibility of a tainted lady transmitting herpes to her tyke amid pregnancy. Second, herpes can make an individual more inclined to HIV contamination which can prompt AIDS [6,7].

2. METHODS OF PREPARATION

2.1. Liquid-liquid suspension polymerization

Microsponge particles are in a liquid-liquid system. This is a process for the oil in the water suspension. It is achieved by the swift stirring of monomers that are liquid (the oil phase). Droplets of these monomers are formed and are stabilized by the dispersing agent. Sometimes the monomer mixture may also contain a polymerization initiator; in that case, a porous polymer is produced as the product. Polymerization is established with distinct droplets of the required size. Polymerization is affected by activating the monomers either by catalysis, increased temperature or irradiation [8,9]. As the polymerization process continues, a spherical structure is formed containing thousands of microspheres bunched together like grapes, forming interconnecting reservoirs in which the porogen is entrapped. These reservoirs open onto the surface of the spheres through which the active ingredient can be released when triggered. Particles forming polymerization mixtures are usually two-phase systems. The monomers are referred to as the 'monomer phase' or 'dispersed phase'; the immiscible liquid phase containing the dispensed (or dissolved) monomer is defined as 'Polymerization medium.' Polymerization takes

place in the presence of 'porogens' or "monomer diluents". This liquid miscible is with the monomer and immiscible with the medium or formed polymers. Thus, as polymerization proceeds, pores are formed into the spaces where porogens are formed. Porogens belong to the category of inert; non-polar organic solvents when added to the polymerization reaction, polymeric beads with open, porous structures can be obtained and they look just like sponges under SEM, hence the name 'micro sponge'. Once polymerization is complete the solid. The particles are then washed and processed until they are substantially ready for use. Particle formation and incorporation of the functional substance is thus performed as a single step. This may be termed a one-step process [10].

2.2. Quasi-Emulsion solvent diffusion

Microsponges are prepared by quasi-external phase and internal phases are used. The internal phase is the organic phase containing drug, ethyl alcohol/acetone (good solvent), polymer and triethyl cit (TEC)/Trichloromethane /Dichloromethane (bridging liquid), which is added to facilitate the plasticity. The external phase mostly consists of distilled water and polyvinyl alcohol (PVA). Weighed amounts of drug and polymer are dissolved in me IPA/ DCM. The formed solution is poured into water containing polyvinyl alcohol. Ethanol/ IPA/ DCM and drug and polymer mixture solution were finely dispersed in the aqueous phase as discrete droplets of the polymer solution of the aqueous phase via counter diffusion of ethanol and water out of and into the droplets. The formed microparticles are filtered and washed with distilled water before being tray-dried at room temperature [11].

2.3. Screening for components of microemulsion

Most important criterion for screening of components for microemulsion formulation is the solubility of the drug in oils, surfactants and co-surfactants. Solubility of Acyclovir in various oils, surfactants and co-surfactants was determined by dissolving the excess amount of drug in 2 mL of each selected oil, surfactants and co-surfactants in a 5 mL capacity stoppered vials separately and mixing using vortex mixer. Mixture vials were kept at 25 ± 1.0 °C in an isothermal shaker for 72 h to reach equilibrium. Equilibrated samples were removed from the shaker and centrifuged at 5000 rpm for 15 min, supernatant was taken and filtered through0.45 µm membrane filter. The concentration of Acyclovir was determined in each component by double beam UV-VIS spectrophotometer at λmax251 nm after appropriate dilution with methanol [12,13].

2.4. Construction of pseudo ternary phase diagram

To determine the concentration of components for the existing range of formulation, a pseudoternary phase diagram will be constructed using the water titration method at ambient temperature (25°C). Surfactant and co-surfactant will be mixed in different volume ratios and titrated with water by dropwise under gentle agitation. The proper ratio of one excipient to another in Microemulsion formulation will be analysed and a pseudo ternary plot will be constructed using TRIPLOT V14 software. All studies will be repeated three times, with similar observations being made between repeats [14,15].

2.5. Optimization of oils, surfactants and co-surfactants

From pseudo ternary phase diagrams showing the maximum microemulsion area, the number of microemulsions with different formulas was selected covering the entire range of microemulsion occurrence in the phase diagram. For each phase diagram constructed, a different formulation was selected from the microemulsion region so that the drug could be incorporated in the oil phase on the following bases. (a) Oil concentration should be such that it.

Table: List of materials and sources

Ingredients	Materials	Source
Drug	Acyclovir	Glenmark Pharma, Mumbai
Oils	Sunflower oil, Castor oil, Oleic acid, Isopropyl myristate	Himedia Pvt. Ltd, Mumbai
Surfactants	Labrasol, Tween 80, Tween 20	Sulab Pioneer, Baroda
Co-surfactants	Propylene Glycol, Polythylene glycol 400	Sulab Pioneer, Baroda

2.6. Formulation and development of Acyclovir microemulsion using Design of experiments [DoE] approach

A design space can signify formulation and process understanding viz. attributes that are related to drug substance, materials, equipment, IP and finished product quality. For this purpose, risk assessment was done based on understanding the process and formulation-related parameters of microemulsion quality. Preliminary studies and later Design of Experimentation (DoE) were carried out for high-risk parameters. Based on the effect of critical quality attributes of the target product profile, we proposed design space for obtaining robust formulation. Characterization of microemulsion will be done for various parameters like the effect of globule size, viscosity, %transmittance, and %drug release [16,17].

3. CHARACTERIZATION OF ACYCLOVIR-LOADED MICROEMULSION

3.1. Droplet size analysis

Droplet size analysis of microemulsion was measured by diffusion method using light scattering, particle size analyser counter, LS 230. It is also measured by correlation spectroscopy that analyses fluctuation in the scattering of light due to Brownian motion. Droplet size analysis of microemulsion can also be performed by transmission electron microscopy (TEM).

3.2. Dilution test

Dilution of microemulsion either with oil or with water can reveal this type. Test is based on fact that more of continuous phase can be added into microemulsion without causing problem of its stability. Thus o/w microemulsion can be diluted with water and w/o microemulsion can be diluted with oil.

3.3. Zeta potential

Zeta potential is measured by an instrument known as Zeta PALS. It is used to measure charge on the surface of droplets in microemulsion.

3.4. pH

The apparent pH of the formulation will be measured by a pH meter. 5.11.6 Density measurement: Density was determined by using a pycnometer. Take the empty weight of the picnometer. Microemulsion was taken in a pycnometer and weight was determined by using an electronic balance. Now difference between total weights and empty picnometer weight would give weight of the formulation. The density of the formulation was calculated by formula. The morphology and structure of microemulsion are studied using transmission electron microscopy. Combination of bright field imaging at increasing magnification and of diffraction modes is used to reveal form and size of microemulsion droplets. Observations are performed as drop of microemulsion is directly deposited on holey film grid and observed after drying.

3.5. Viscosity determination

Viscosity of Microemulsion will measure by using Brookfield-type rotary viscometer at different shear rates at different temperatures.

3.6. In-vitro drug release studies

Drug release kinetics was studied using modified method glass cup with cross-sectional area of 1.5 cm2 will fill with 0.2ml of microemulsion, cover with cellophane membrane, seal with rubber band and adhesive tape, and invert under surface of 30

ml of phosphate buffer pH in USP XXIII Type I Dissolution Test Apparatus with speed of 30 rpm. 1ml of aliquots will be withdrawn at specified time intervals and immediately replaced with fresh dissolution medium. Drug content in withdrawn samples will be determined spectrophotometrically at 251 nm.

3.7. Thermodynamic stability studies

During thermodynamic stability of drug loaded Micro-emulsions following stress tests as reported:

a. Heating Cooling Cycle: Microemulsion formulations will be subjected to six cycles between refrigerator temperature (4°C) and 45°C. Stable formulations were then subjected to centrifugation test.

b. Centrifugation: Microemulsion formulations will be centrifuged at 3500 rpm and those that did not show any phase separation were taken for freeze thaw stress test.

c. Freeze Thaw Cycle: In this formulation will be subjected to three freeze thaw cycles between 21°C and +25°C kept under standard laboratory conditions. These studies will be performed for period of 3 months.

3.8. STABILITY STUDIES AS PER ICH GUIDELINES

Stability studies of microsponges as per ICH guidelines: The stability of an active pharmaceutical in the finished pharmaceutical product can be assessed by the stability studies as per the ICH guidelines. The ICH has prescribed the specifications under which the stability studies are to be conducted.

Table: Stability studies as per ICH guidelines

Study type with duration	Storage Condition
Long term/ real time	25° C/60% RH
Intermediate	30 ° C/65% RH
Accerated	40 ° C/75% RH

4. RESULTS AND DISCUSSIONS

4.1. Droplet size analysis of optimized batch

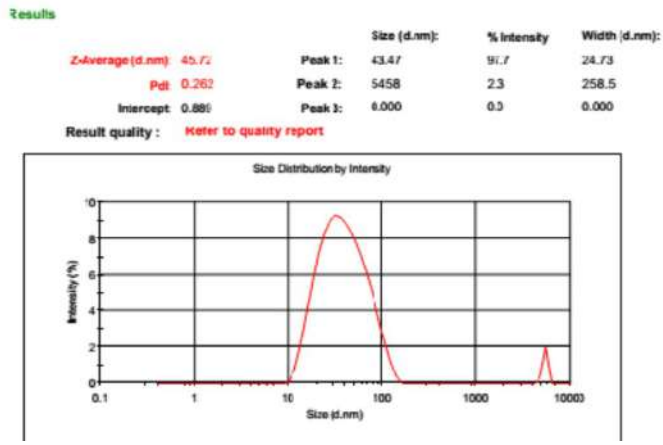

Figure 6.44: Droplet size analysis of Optimized Batch

The Main feature of microemulsion is strict droplet size, which must be in the micrometer range. Therefore, size analysis was performed to confirm whether the resultant emulsion was indeed a microemulsion. The average droplet size of microemulsion was found to be 45.72 nm. Small droplet sizes are very much a prerequisite for drug delivery as oil droplets tend to fuse with skin thus providing a channel for drug delivery.

4.2. Dilution test of microemulsion formulation

Table 6.49: Dilution test of microemulsion

Dilution	Observation
10	No phase separation
50	No phase separation

100	No phase separation

Prepared microemulsion formulation was diluted in 1:10, 1:50, 1:100 ratio with distill water system don't show any sign of separation and found to be clear. So,it's concluded that prepared microemulsion is o/w type.

4.3. Measurements of zeta potential

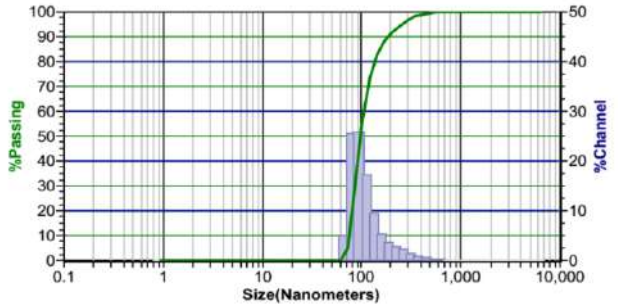

Figure 6.45: Zeta potential of Optimized Batch

Table: Zeta potential measurement

Mobility	0.08 u/s/V/cm
Zeta Potential	27.5 mv
Charge	0.002 cfu
Polarity	Negative
Conductivity	1156 uS/cm

Zeta potential of Acyclovir microemulsion was found to be 27.5. Zeta potential is used to identify charge of oil globules in emulsion. Increase in electrostatic repulsive forces between globules prevents coalescence of microemulsion. On contrary, decrease in electrostatic repulsive forces can cause phase separation. Negative value of zeta potential may be due to presence of fatty acid. Negative value of zeta potential of optimized formulation indicated that formulation was negatively charged and sufficient repulsion among emulsion droplet existed to form un-coagulated stable system.

4.4. Polydispersity index

Polydispersity index indicates the uniformity of droplet size within the formulation and its stability. PDI is a measurement of particle homogeneity and it ranges from 0.0 to 1.0. The value of PDI was found to be 0.262. The low value of PDI indicated the uniform distribution of Microdroplets within the formulation.

4.5. Refractive Index:

The refractive index is the net value of components of the microemulsion and indicates the isotropic nature of the microemulsion. The mean value of the refractive index for the formulation was found to be 1.33

4.6. pH

pH value for optimized drug-loaded microemulsion was recorded to be 6.5 which is favourable for topical application since pH of the skin ranges from 5.5 to 7.0.

4.7. Thermodynamic Stability

Table 6.53: Thermodynamic Stability study

Batch	Heating cooling cycle	Centrifugation	Free Thaw cycle
ACME10	+	+	+

4.8. In-vitro drug release study:

Table 6.54: In-Vitro Drug Release study

Time (hrs)	% Drug release
0	0
1	66.63
2	75.56
3	88.62

CONCLUSION

Drug delivery via polymer systems has been proposed to be the prevailing type of controlled drug delivery device both in present and future. For scientific as well as economic reasons, such delivery systems have potential advantages which include enhanced therapeutic response, predictable rate of release and extent of absorption and improved patient acceptance. The present study explores the making of microsponges of

Acyclovir that can be delivered orally in a controlled manner to improve efficacy and patient compliance. In the present study DoE by QbD approach has been applied as the tool for the development of Acyclovir microsponges. In the start preliminary study of drugs and excipients was done. Microsponges were prepared by the Quassi-emulsion diffusion method. Then the selection of internal phase, external phase, polymer and surfactant were done. Then variables like volume of internal phase, volume of external phase, concentration of surfactant, drug: polymer ratio, speed of stirring and time of stirring were studied. Then preliminary batches were prepared and evaluated for their % yield, drug content, loading efficiency, particle size etc. Identification of independent and dependent variables was done. After setting of QTPP and CQAs the DoE by QbD was applied. 23 Full factorial design was applied for DoE. All the batches were manufactured and evaluated for their characteristics. Finally, the validation batches were manufactured and evaluated and stability studies were conducted. Microemulsions are a promising way of delivering the drugs via the topical route. In the present study, efforts were taken to formulate the microemulsions of the drug Acyclovir into a suitable microemulsion dosage form to enhance patient compliance. For the formulation of microemulsion first, the drug and various excipients were subjected to preformulation evaluation. After that, the excipients were screened which were to be used for preparation of microemulsion. Preliminary trial batches were manufactured and evaluated. Then DoE by QbD approach was applied as the tool for the development of Acyclovir microemulsion. Identification of independent and dependent variables were done. After setting of QTPP and CQAs the DoE by QbD was applied. 32 Full factorial designs were applied for DoE. All the batches were manufactured and evaluated for their characteristics. Finally, the validation batches were manufactured and evaluated and stability studies were conducted. From the work, it can be concluded that microsponge and microemulsion are promising drug delivery systems for Acyclovir. They have the potential to increase efficacy and improve patient compliance for sure.

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