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Formulation and Evaluation of Nanoemulsion based Transdermal Patch for Acyclovir

Archana SP*, Abhilash J, Swarupeshwari RP, Yadishma G and Sagar P

Department of Pharmaceutics, India

***Corresponding author:** Archana S Patil, Associate Professor, Department of Pharmaceutics, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, Karnataka-590010, India, Tel: +91-9916883344; Email: archanapatil@klepharm.edu

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Abstract

The effectiveness of Acyclovir ointment in treating herpes simplex virus infections can be limited by its moderate efficacy, particularly in severe cases. This limitation is partly attributed to the slow absorption of the drug through the skin's epidermal layer. Consequently, there is considerable interest in developing improved formulations to enhance the topical delivery of Acyclovir. Nanoemulsions, colloidal dispersions of oil and water stabilized by surfactants, offer a promising approach to improve drug penetration.

In this study, we aimed to develop and characterize a nanoemulsion-based transdermal patch for enhanced delivery of Acyclovir. Screening of surfactants, oils, and co-surfactants based on solubility studies guided the formulation process. Pseudoternary phase diagrams helped determine optimal concentrations of Smix and oils for nanoemulsion preparation. Three formulations were evaluated for particle size, encapsulation efficiency, and zeta potential, with the optimized formulation selected for incorporation into the transdermal patch.

The patch was formulated using 4% polyvinyl alcohol and 1% polyvinyl pyrrolidine as suitable polymers. Evaluation of drug content and diffusion across a membrane confirmed successful incorporation of the nanoemulsion. In vitro release studies demonstrated zero-order release kinetics with sustained drug release over 2 hours for all formulations and up to 7 hours for the patch. Importantly, the nanoemulsion-based transdermal patches exhibited enhanced drug diffusion and permeation compared to pure drug formulations.

Overall, this study highlights the potential of nanoemulsion-based transdermal patches as a promising strategy for improving the delivery of Acyclovir, offering extended release, reduced administration frequency, and enhanced therapeutic efficacy in treating herpes simplex virus infections.

Keywords: Acyclovir; Herpes; Transdermal Patch; Nanoemulsion

Abbreviations: HSV: Herpes Simplex Virus; ACV: Acyclovir; BCS: Biopharmaceutical Classification System; RPM: Revolutions Per Minute: PTFE: Polytetrafluoroethylene.

Introduction

HSV infections are widespread globally, affecting approximately 67% and 13% of the human population. These viral infections persist throughout life and are characterized by recurrent flare-ups at the original infection site. HSV type 1 typically spreads through oral contact, often resulting in cold sores, as well as less common conditions like keratitis, ocular complications, and encephalitis. HSV type 1 genital infection, transmitted via oral-genital contact, is increasing, although reactivations occur less frequently compared to HSV type 2 [1-4].

HSV undergoes two replication cycles: the lytic cycle, where it produces infectious viral particles to infect other cells, and the latent cycle, characterized by minimal gene expression and the absence of infectious virus particles. The outcomes of HSV-1 and HSV-2 infections vary from asymptomatic to mild or potentially life-threatening. While most immunocompetent individuals experience mild, self-resolving symptoms, HSV infections can lead to significant morbidity and mortality in certain cases, with exact reasons not entirely understood [5,6].

Acyclovir (ACV) is a type of antiviral medication belonging to the guanosine class. It's widely prescribed for treating infections caused by the herpes simplex virus. Acyclovir belongs to BCS class III which has high solubility and low permeability, having low oral bioavailability of about (10%-30%) [7-9]. The utilization of ACV has experienced significant growth in recent times owing to its effectiveness in managing diseases caused by HSV infection, along with its application in combination with various other pharmaceutical medications for treating a range of infections [10].

Nanoemulsion also known as submicron emulsion, ultrafine emulsion and mini emulsions. This are submicron sized colloidal particulate system considered as thermodynamically and kinetically stable isotropic dispersions, which consist of two immiscible liquids like water and oil, stabilized by and interfacial film consisting of a suitable surfactant and co- surfactant to form a single phase. Nanoemulsions more recently are classified into three categories such as O/W, W/O and bi- continuous [11].

The currently available acyclovir topical formulations such as creams, gels, patches have low efficacy due to poor percutaneous penetration which reduces the amount of the drug required to reach the basal epidermis to prevent the reactivation of dormant viruses responsible for recurrence of infection. Topical application of drug may cause burning and stinging sensations.

The study focuses to formulate nanoemulsion based transdermal patch of Acyclovir for the treatment of HSV infection. Due to the drug's low oral bioavailability, nanoemulsions were developed to enhance the bioavailability of poorly water-soluble drugs. These formulations not only improve the pharmacological and therapeutic effects of the drug but also reduce its adverse and toxic effects. Formulation of nanoemulsion based transdermal patch will administer a therapeutic dose of medication through the skin, facilitating systemic effects for healing targeted areas of the body. Compared to alternative delivery routes like oral, intravenous, topical, or intramuscular methods, transdermal patches offer the advantage of controlled medication release, typically achieved through a porous membrane containing a medication reservoir or via body heat, which melts thin layers of medication within the adhesive [12,13].

Materials and Methodology

A 97% pure standard Acyclovir was procured from Zydus Cadila Healthcare Ltd. The research employed Cremophor Rh from BASF care creations, Sodium hydroxide pellet from S. D fine chem Limited, Oleic acid from Adani wilmar Ltd, PVP from Himedia laboratories pvt. Ltd, PVA from S.D fine chem Limited, Propylene glycol from Loba chemie Pvt. Ltd, Potassium dihydrogen orthophosphate from S.D fine chem Limited.

Preparation of Calibration Curve for Acyclovir

Preparation of Stock Solution of Acyclovir in Buffer pH 7.4: Take 100mg of Acyclovir in require quantity of buffer pH 7.4 in 100ml of volumetric flask and make up the volume to 100ml using the same buffer (Stock solution A). Withdraw 1ml of stock solution A in 10ml of volumetric flask make up the volume to 10ml using buffer of pH 7.4 (stock solution B) from the above stock solution B the dilution is made for 0.2ml, 0.4ml, 0.6ml, 0.8ml, and 1ml.and the absorbance is taken at 252nm [14].

Identification of Acyclovir by DSC: To examine the interaction between the drug and excipients. The acquired thermogram endothermic peck was compared with endothermic peak of the Acyclovir pure drug.

Preparation and Evaluation of Nanoemulsion

Screening of Oils, Surfactants, and Co-surfactants: To determine the best oil, surfactant, and co-surfactant for Acyclovir, solubility studies were conducted. Excess amounts of Acyclovir were added to 5 ml of each oil, surfactant, and co-surfactant in stoppered vials. These vials were initially mixed and then placed on a magnetic stirrer for 24 hours at room temperature to reach equilibrium. After this period, the mixtures were centrifuged at 10,000 rpm for 10 minutes.

The supernatant was separated and filtered through PTFE (Polytetrafluoroethylene) syringe filters with a 0.45µm filter disk. The filtered solution was then diluted with methanol, and its absorbance was measured at a wavelength of 252 nm. This analysis determined the solubility of Acyclovir in different oils, surfactants, and co-surfactants, helping in the selection of suitable components for further formulation development.

Development of Pseudo Ternary Phase Diagrams: To confirm the presence of a nanoemulsion region, pseudo-ternary phase diagrams were created. Oleic acid was chosen as the oil phase, Cremophor Rh as the surfactant, and propylene glycol as the co-surfactant due to their high solubility with Acyclovir. The phase diagrams were made using the aqueous titration method without the drug, adding water in small amounts. The surfactant and co-surfactant were mixed in different ratios (0.5:1.2, 0.2:0.4, 0.4:1, 0.8:1, 1.2:0.6, 1.2:0.5, 1:0.8, 0.9:0.4). Each surfactant/co-surfactant mixture (Smix) was then combined with the oil in various ratios (oil: Smix) of 0.26, 0.4, 0.5, 0.1, 0.1, 0.21, 0.17, and 0.5. These oil and Smix mixtures were vortexed to form a uniform blend. Distilled water was then added gradually until turbidity appeared, indicating phase formation. Visual observations were recorded for each phase diagram. These pseudo-ternary phase diagrams helped identify the right compositions for the nanoemulsion region, aiding in the formulation of stable and effective nanoemulsions.

Trial No	Oil	Surfactant	Co-surfactant	Water consumed (ML)
1	12.8789	27.1211	60	22.1
2	20	60	20	17.8
3	25.3703	20	54.6297	6.8
4	5	40.5647	54.4353	24.7
5	9.125	60	30.875	6.8
6	12.8789	60	27.1211	4.7
7	5	52.4739	42.5261	4
8	27.3309	47.7445	24.9247	3

Table 1: Formulation Table of Nano Emulsion.

Formulation of Nanoemulsions

Based on the desired qualities like transparency and stability, three different points within the best areas of the phase diagram were chosen. The amounts of oil, Smix, and water at these points were used to create the formulations. By choosing different centroidal points, it is possible to explore and evaluate the effect of varying compositions on the properties of the formulations, allowing for optimization and customization based on specific requirements.

A small amount of Acyclovir was weighed and dissolved in a measured volume of Oleic acid along with the specified amount of propylene glycol. The mixture was continuously stirred using a magnetic stirrer to ensure it was well-mixed. Cremophor Rh (a surfactant) and water were mixed together to create "Mixture B," which was thoroughly stirred to blend properly. Mixture B was then added slowly, drop by drop, into Mixture A (which contained the dissolved drug, oil, and co-surfactant) while keeping it continuously stirred. This slow addition helped achieve an even dispersion and prevented the formation of large droplets. The resulting mixture was then homogenized at high speed using a homogenizer set to 15,000 rpm for about 15 minutes. This step was essential for reducing droplet size, improving emulsion stability, and creating a clear nanoemulsion. After homogenization, the emulsion was visually checked for clarity and uniformity. Additional tests, such as droplet size analysis and stability studies, were carried out to evaluate the quality and performance of the nanoemulsion formulation [15].

Evaluation of Nanoemulsion

pH Analysis: pH of nano emulsion was determined at room temperature using digital pH meter.

Homogenization: The 2ml of samples was placed in Eppendorf tube and then nano emulsion was centrifuged at 10,000rpm for 30min at room temperature and observed for homogeneity.

Drug Content Nanoemulsion: An accurately 0.1ml of each formulation of oil in water (O/W) nanoemulsion of Acyclovir was placed in 10ml of volumetric flask and diluted to the mark with methanol and absorbance is taken at 252 nm.

Particle Size: Smaller the size of the particle, better the efficiency of drug delivery to the desire site. And also, emulsion droplets may lead to more rapid absorption and improve the bioavailability. it was carried out for base of nanoemulsion and was found to be 0.1- 0.4 (mean diameter). Particle size of nanoemulsion was found to be in the range of nanoemulsion.

Zeta Potential: The droplet surface charge was determined by photon correlation spectroscopy using zetasizer. The presence of charge on surface determines that stability of the product. It is important for the formulation to have same charge as opposite charges attracts which can causes agglomeration of the formulation. The formulation (0.1ml) was dispersed in 50ml of water in volumetric flask, mix thoroughly with vigorous shaking. The light scattering was monitored at 25°C at 90° angle. This angle is also used to identify the charge of the droplets. Higher the polydispersity index, wider the droplet size distributed. Zeta potential determines the stability of the formulation [15-17].

Formulation of Patch

The transdermal patches were formulated by solvent casting method using aluminium foil as a backing membrane. For the preparation of patch mixture of 4% PVA used as backing membrane (polyvinyl alcohol) in 10ml of water. And 1% of PVP used as film former (polyvinyl pyrrolidine), 10ml of ethanol, and 1.5ml of propylene glycol as plasticizer were dissolved properly. For the above mixture add the formulation of nanoemulsion and stirred on a magnetic stirrer until a homogenous mixture was obtained. This mixture was then poured onto petri dish containing aluminium foil allowed to dry at 60 degree centigrade in tray dryer. And the rate of evaporation was controlled by inverting a funnel over the mould. The dried patches were then cut into smaller patches to carry out the evaluation tests [18,19].

Evaluation of Patch

Thickness: The thickness of patch was assessed by using a vernier calliper at different points of patch from each formulation 3 randomly selected patches were used. The average value of thickness of single patch was determined.

Weight: Three patches were taken and weight of each was observed by using electronic balance. The average weight of patch was determined.

% Moisture Content: The prepared film was weighed individually and kept in desiccator containing fused calcium chloride at room temperature for 24hrs. The film was again weighed and the % moisture content was calculated using the formula.

$$\%moisture\,content = \frac{Initial\ weight - Final\ weight}{final\ weight} \times 100$$

In vitro diffusion study

In-vitro diffusion studies were performed using franz diffusion cell with a receptor compartment capacity of 40ml. The dialysis membrane was mounted between the donor and receptor compartment of the diffusion cell. The patch was placed on cellulose acetate membrane and covered with aluminium foil. The receptor compartment of diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a hot plate magnetic stirrer and solution in the receptor compartment was constantly and continuously stirred using magnetic beads and the temperature was maintained at 37°C. The sample were withdrawn at different time intervals and analysed for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal [18,20].

Results and Discussion

Determination of Wavelength Maxima of Acyclovir

The wavelength maxima (λmax) of Acyclovir were found to be 251nm.

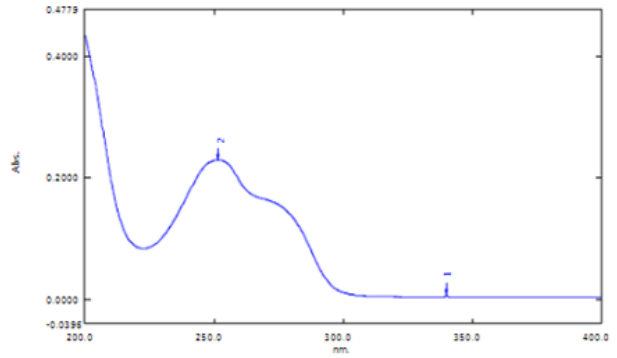

Figure 1: Wave Length Maxima of Drug.

Concentration	Mean Absorbance
0.2	0
0.4	0.165
0.6	0.295
0.8	0.444
1	0.594
1.2	0.748

Table 2: Standard Calibration Curve of Acyclovir.

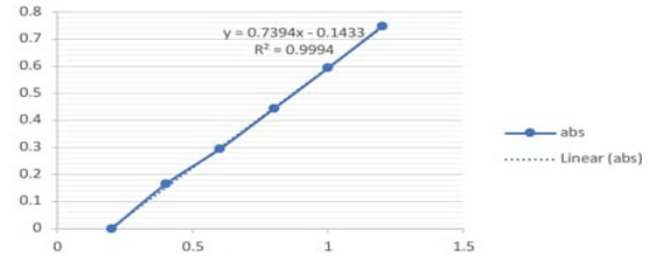

Figure 2: Calibration Curve of Acyclovir.

Identification of Acyclovir by DSC

The obtained endothermic peak of the mixture is shown in Figure 5.

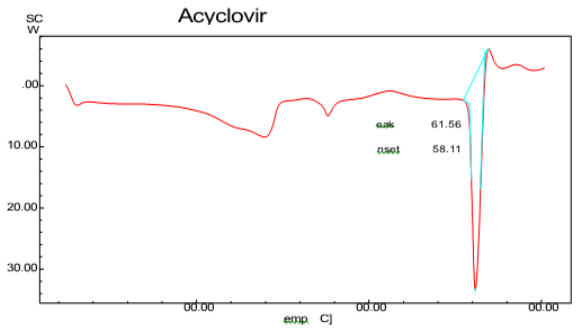

Figure 3: DSC Thermogram of Acyclovir.

Screening of Oils, Surfactant, Co-Surfactant

Selection of oil, surfactant and co-surfactant based on solubility studies. The solubility of Acyclovir in various oils, surfactant and cosurfactant was determined. After solubilising study, oleic acid is selected as oil phase because of drugs highest solubility in it i.e.,0.328 mg/ml. Cremophor RH was chosen as the surfactant, with the drug solubility of 0.700mg /ml, and cosurfactant as propylene glycol with drug solubility of 1.041mg/ml.

Oils	Oleic acid	0.0013
	Liquid paraffin	0.779
	Labrafil 2125	1.087
	Labrafil m 2130	0.226
surfactants	Tween 80	0.421
	Cremophor RH	0.7
	Span 80	0.59
	Tween 20	0.183
cosurfactant	PEG 400	0.109
	PEG 600	0.324
	Propylene glycol	1.041

Table 3: Different Oils, Surfactants and Co-Surfactants Selected for Screening.

Development Pseudo Ternary Phase Diagram

Pseudo ternary phase diagram was constructed to confirm the existence of nanoemulsion region. Oleic acid as oil phase, Cremophor Rh as surfactant and propylene glycol as co-surfactant were based upon the highest solubility of Acyclovir. Pseudoternary phase diagram were constructed by aqueous titration method without drug by incremental addition of water. Smix (Cremophor RH and Propylene glycol) were prepared with different ratios of surfactant and cosurfactant (0.5:1.2, 0.2:0.4, 0.4:1, 0.8:1, 1.2:0.6, 1.2:0.5, 1:0.8, 0.9:0.4) each Smix was mixed with oil (Oleic acid) in different weight (0.26, 0.4, 0.5, 0.1, 0.1, 0.21, 0.17, 0.5). This mixture is then titrated with aqueous medium until turbidity is formed. The red marking in the following psuedoternery phase diagram shows the design space to select the concentrations of oil, surfactant and cosurfactant.

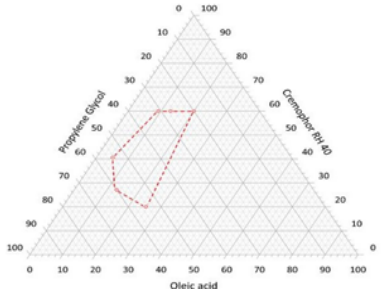

Figure 4: Pseudo-Ternary Phase Diagram.

Formulation of Nanoemulsion

Three different formulations of nano emulsion were prepared with the help of pseudo ternary phase diagram by which different concentration of oils, surfactants and co-surfactants were selected. The formulated nanoemulsions are shown in below Figure 4.

Ingredients	F1	F2	F3
	0.1g	0.1g	0.1g
Oil	5ml	12ml	20ml
Smix	74ml	85ml	60ml
water	20.9ml	2.9ml	19.9ml

Table 4: Formulation Table of Nano Emulsion.

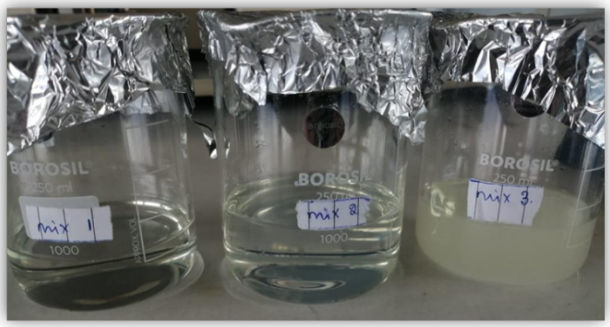

Figure 5: Prepared Nanoemulsion Formulations.

Evaluation of Nanoemulsion

pH Analysis and Homogeneity: The pH of the topical formulations must be compatible with the skin pH. The pH of all the 9 formulations was within the limit from 4.95±0.56 to 5.27±0.49 which is found to be acceptable and avoid the skin irritation.

Percentage Drug Content and Particle Size Determination: The percentage of drug content is more in F1 formulation than the other F2 and F3 formulations and particle size of F1 formulations is within the range that is 152nm so compared to other formulations so, F1 formulation was selected as optimized formulation to incorporate into a patch.

Formulation	Absorbance	% Drug content	pH	Homogeneity	Particle Size
F1(1:14)	0.28	89.99±0.61	4.95±0.56	Homogenous	152±0.97
F2(1:7)	0.24	82.77±0.51	5.27±0.49	Homogenous	175±0.84
F3(1:3)	0. 20	80.20±0.67	5.07±0.81	Homogenous	180±0.63

Table 5: Evaluation Parameters of Nanoemulsion.

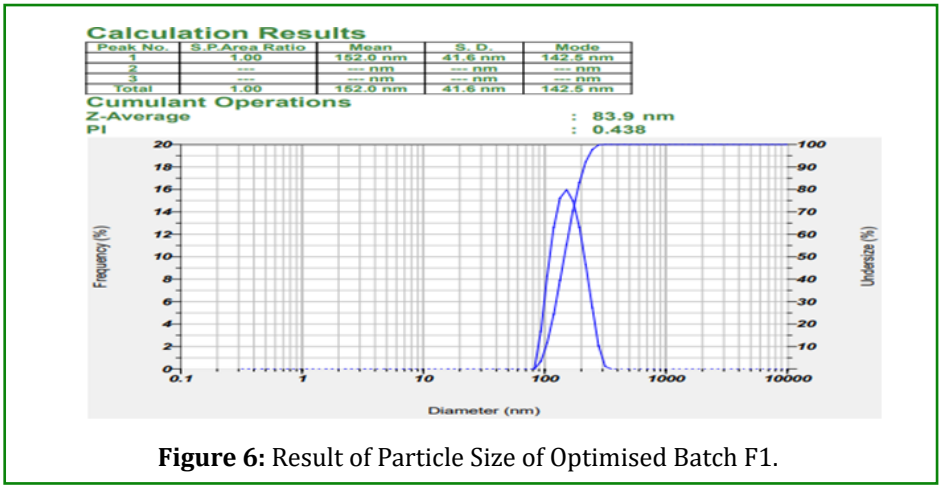

Figure 6: Result of Particle Size of Optimised Batch F1.

Zeta potential of Acyclovir: The Particles with zeta potential, the more negative zeta potential, greater the net charge of droplet and more stable the nanoemulsion. Zeta potential value lower than 30mV generally indicate high degree of physical stability.

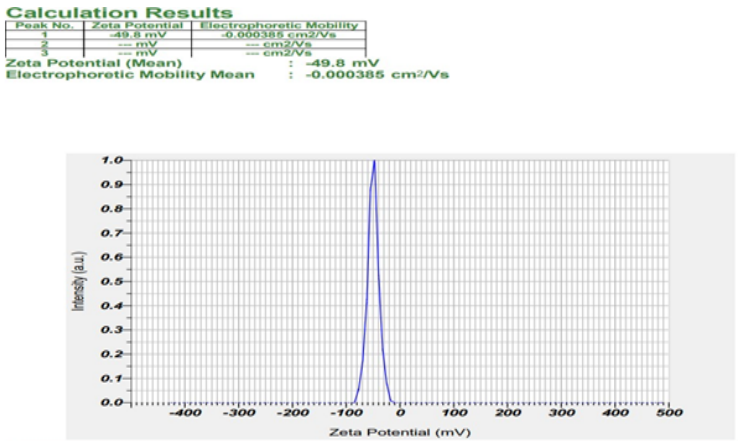

Figure 7: Result of Zeta Potential of Optimised Batch F1.

Evaluation of Patch: The patch was evaluated for weight, thickness and moisture content and the same were given in Table 6.

- Average weight of each 3 patches was 0.39g.
- Average moisture content of each 3 patches was 2.64%.
- Average thickness of each 3 patches was 0.9mm.

F1	Weight	%Moisture Content	Thickness Patch 1 Patch 2	Patch 3
1	0.35g	2.77%	0.75mm 1mm	1mm
2	0.40g	2.50%	1.2mm 1.1mm	1.2mm
3	0.42g	2.65%	0.8mm 0.6mm	0.8mm
			0.9mm 0.8mm	0.7mm
		Mean:	0.9mm 0.87mm	0.8mm

Table 6: Various Evaluation Parameters for Patch.

Figure 8: Prepared Nanoemulsion Based Transdermal Patch.

***In Vitro* Diffusion Study:** The *in vitro* drug release profiles of all the formulations have been shown in figure 11. The release of Acyclovir mainly depends upon the polymer concentration. The release rate of the drug from the patch was found to decrease drastically with increase in polymer concentration. The polymers used are PVA and PVP of varying concentration. *In vitro* release studies showed zero-order release of the drug from all the patches, and the mechanism of release was diffusion mediated. Moreover, the release of the drug was sustained and it extended over a period of 2hr in all formulations. Whereas the release of the patch over a period of 7hr. The study has achieved the objectives of transdermal drug delivery system, such as avoidance of first pass effect, extended release, and reduced frequency of administration. Overall, the studies implicated that the NE based carrier contributed significantly to the enhancement of the membrane permeation. The nanoemulsion technology with reduced particle size provided more surface area that improved the solubility of Acyclovir as well as permeation.

Time	Absorbance	Concentration	mg/1ml	mg/900ml	% Drug Release
0	0	0	0	0	0
1	0.079	2.891	0.065	0.0659	5%±0.58
2	0.088	2.891	0.0792	0.0792	10%±0.91
3	0.12	3.94	0.108	0.108	35%±0.64
4	0.14	4.263	0.111	0.122	47%±0.56
5	0.15	5.222	0.213	0.235	60%±0.89
6	0.17	6.123	0.233	0.333	71%±0.68
7	0.188	7.124	0.245	0.358	80%±0.81

Table 7: Percentage Drug Release of Patch (n=3).

Time(min)	Absorbance	Concentration	mg/ml	mg/900ml	% Drug Release
0	0	0	0	0	0
1	0.11	71	1775	63.9	80%±0.91
2	0.152	99	2475	85	97%±0.85

Table 8: Percentage Drug Release of Pure Drug (n=3).

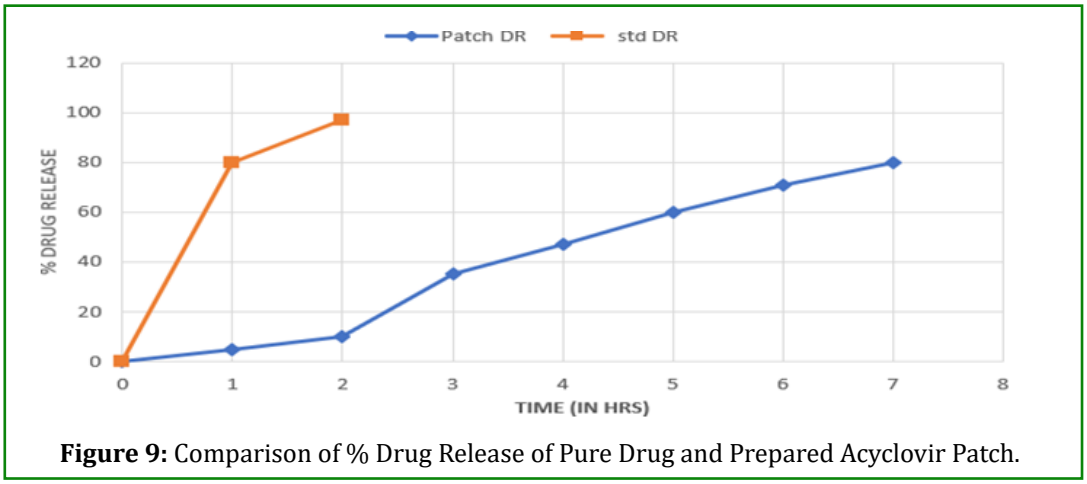

Figure 9: Comparison of % Drug Release of Pure Drug and Prepared Acyclovir Patch.

Conclusion

The present study successfully developed a transdermal patch of Acyclovir. This study showed that nanoemulsion based transdermal patch of Acyclovir can be used to improve drug aqueous solubility and permeability through biological membrane. Screening of surfactant, cosurfactant and oil was done and most suitable excipient is identified. Whereas the ternary phase diagram gave an idea about the concentration range of excipient that should be employed to achieve stable nanoemulsion formulation. Nanoemulsion was prepared by using high pressure homogenization process. Based on the evaluation studies of nanoemulsion optimised one was selected to incorporate into the patch. On the basis Acyclovir research paper and pre formulation study for different parameters was done such as identification of drug, solubility, melting point and calibration curve.

Nanoemulsion incorporated transdermal patch of Acyclovir drug was successfully formulated by solvent casting method by using polyvinyl pyrrolidine, polyvinyl alcohol and propylene glycol as a polymer of varying concentration. Prepared patches were found to have smooth and uniform surface. This patch was then evaluated for various parameters. The drug content in each 2cm patch was found to be 0.125mg/ml. The *in vitro* drug release study showed that drug release was found to have 95% in 7hrs from the patch and the drug release of pure drug was found have 97% in 2hr.

Hence it was noticed that there was enhanced drug diffusion/ permeation of nanoemulsion based transdermal patch than the pure drug. The study has achieved the objectives of transdermal drug delivery system, such as avoidance of first pass effect, extended release, and reduced frequency of administration.

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