RESEARCH ARTICLE

Cold Emulsified Nanoemulsion: Enhancing Delivery and Stability of Thermolabile Acyclovir

P Bhatt*, P Bigoniya

DSKM College of Pharmacy, RKDF University, Bhopal, Madhya Pradesh, India.

Received: 08th September, 2023; Revised: 29th October, 2023; Accepted: 05th January, 2024; Available Online: 25th March, 2024

ABSTRACT

Acyclovir is an acyclic purine nucleoside widely used as an antiviral agent with good topical efficacy. Acyclovir is a thermolabile drug, making it challenging to formulate a stable and effective emulsion via a conventional emulsification process. This study aims to develop, optimize, and characterize the nanoemulsion of thermolabile drug acyclovir, manufactured by adopting a cold emulsification process and compare the physicochemical properties and diffusion pattern with a leading marketed formulation. Diffusion studies were conducted using Franz diffusion cells. The optimized formulation was subjected to quantitative estimation of acyclovir, impurity profiling, viscosity, pH, vesicle size, shape, and polydispersibility index (PDI). In the current study, the particle size of the nanoemulsion varies from 66.25 to 244.40 nm, and the zeta potential revealed a high negative surface charge on the particles. Transmission electron microscopy images revealed spherical-shaped, non-aggregated, and discrete globules. The *in-vitro* diffusion study of acyclovir nanoemulsion showed enhanced penetration efficacy than the conventional emulsion. All these observations signified that the cold emulsification process for manufacturing nanoemulsion is appropriate for thermolabile and pH-sensitive drugs. The study demonstrates enhanced stability and efficacy of optimized acyclovir nanoemulsion through cold emulsification.

Keywords: Cold emulsification, Nanoemulsion, Stability, Acyclovir, Zeta potential, *In-vitro* diffusion, Transmission electron microscopy.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.25

How to cite this article: Bhatt P, Bigoniya P. Cold Emulsified Nanoemulsion: Enhancing Delivery and Stability of Thermolabile Acyclovir. International Journal of Drug Delivery Technology. 2024;14(1):174-178.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Topical drug delivery is always a preferred route of administration where the action is intended locally since the skin is the superficial and largest part of the body.[1] The nanoemulsion gel formulations exhibit higher viscosity and are more stable than conventional nanoemulsions.[2,3] Acyclovir is an acyclic purine nucleoside antiviral for topical application and the onset of action starts immediately at the site of application.[4] Acyclovir is thermos sensitive and is prone to degrade at lower and higher pH solutions. It is difficult to stabilize acyclovir in a conventional emulsification process and retain its potency during the product's shelf life. The pH and process temperature need to be optimized to formulate a stable acyclovir topical dosage form.[5]

The present study aimed to formulate a cold emulsified nanoemulsion gel of thermolabile drug, acyclovir through temperature and pH-independent process to provide a stable formulation. Nanoemulsions of acyclovir were formulated using a cold emulsification process to control the impurity profiling of the finished product and enhance the absorption rate. The optimized acyclovir nanoemulsion formulations were characterized and validated for globule size, zeta potential, morphology, and *in-vitro* absorption profile and were compared to a reference product.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grades

Characterization of Acyclovir

The characterization was performed as per[6]

Identification, assay and impurity profiling

The identification, assay, and impurity profiling were done using the following procedure:

- *Solubility*

Solubility determination of acyclovir was performed as per.[7]

- *Compatibility of acyclovir with excipient*

Compatibility studies of acyclovir and excipients were conducted as per.[8]

*Author for Correspondence: pankaj.bhatt@outlook.com

- *Optimization of nanoemulsion gel*

Optimization of process time was done using 4M, 6F, and 8SF configurations.[9]

- *Acyclovir-loaded nanoemulsion gel*

For the preparation of acyclovir-loaded nanoemulsion, the cold emulsification process was used as mentioned.[10]

- *Process optimization and comparison with marketed formulation*

After prototyping and finalization of the formula, process optimization for homogenization was performed, and batches were compared for physicochemical parameters.[10]

Formulation Characterization

Quantification

Acyclovir content and impurity profiling (Guanine) of nanoemulsion gel was performed following the validated high-performance liquid chromatography (HPLC) method as described above.[6]

Refractive index

Refractive index was performed according to.[11]

Viscosity

The viscosity of all the optimized formulations was determined using a BrookField viscometer.[10]

Vesicle size

Malvern P analytical was used to determine vesicle size in micrometer.

Zeta potential

The zeta potential of samples was measured by Zeta sizer Ver-7.12 (Malvern Zetasizer, UK).

Polydispersibility index

Malvern Panalytical was used to determine Polydispersibility index (PDI).

Morphology

Transmission electron microscopy (TEM) was performed as per.[12]

In-vitro skin permeation

In-vitro concentration of acyclovir released was determined by HPLC.[10]

RESULTS AND DISCUSSIONS

Characterization of Acyclovir

Acyclovir is a white, crystalline, odorless powder showing a melting point of 256 to 258°C. The assay of acyclovir was found to be 99.7%, which was as per the limit specified in the USP monograph. The IR spectrum of acyclovir shows principal absorption peaks at wave number 3438, 3176.61 cm^{-1} for OH (Phenolic) and NH_2, respectively and 1709.49 cm^{-1} for ester C=O stretching. The IR spectra results conform to the structure of acyclovir as per the reference standard. Acyclovir and nanoformulations were analyzed for assay and impurity profiling (Guanine) through the validated HPLC method that potentially showed the absence of any significant impurity (Figure 1).

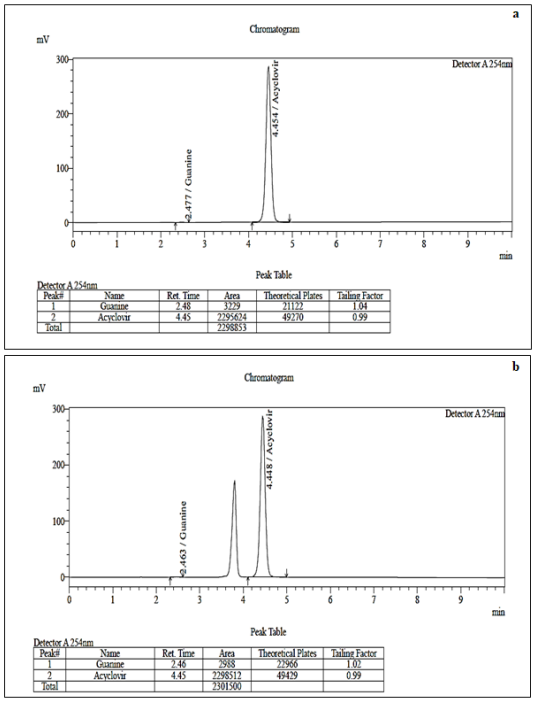

Figure 1: HPLC estimation of acyclovir sample (a) and optimized nanoemulsion PB-020 (b)

Solubility

The solubility of acyclovir was ascertained in different media. On the basis of the solubility studies, it was concluded that acyclovir has higher solubility in surfactant and emulsifier systems (Table 1).

Compatibility of Acyclovir with Excipient

Acyclovir did not show degradation in compatibility studies while analyzing the solution at the last station (4th week

Table 1: Solubility of acyclovir in different solvents, emulsifiers and co-emulsifiers

Blend process	*Solvent*	*Solubility*
Acyclovir was mixed with different solvents 100 mg in 10 mL and mixed in an orbital shaker for 6 hours. Conducted at 25–27°C	Water	Slightly soluble
	Methanol	Insoluble
	Ethanol	Insoluble
	Isopropyl alcohol	Insoluble
	Dimethyl formamide	Slightly soluble
	Propylene glycol	Highly soluble
	Polyethylene glycol	Highly soluble
	Acidic solution of citric acid	soluble
	Basic solution of NaOH	Insoluble
	Tween 80	Soluble
	Crempphor RH 40	Highly soluble
	Tween 20	Highly soluble
	Transcutol	soluble

Table 2: Compatibility of acyclovir with proposed excipients

Sample (Acyclovir + Excipients)	*Ratio*	*Initial description*	*40°C/75%RH*				*4°C*
			1 Wk	*2 Wk*	*3 Wk*	*4 Wk*	*4 Wk*
+Acrylamide/Sodium acryloyldimethyl taurate Copolymer/Isohexadecane & Polysorbate 80	1:4	Off-white	-ve	-ve	-ve	-ve	-ve
+Mineral oil/Sunflower oil	1:4	Off-white	-ve	-ve	-ve	-ve	-ve
+PEG-40 Hydrogenated castor oil	1:4	Off-white	-ve	-ve	-ve	-ve	-ve
+Propylene glycol	1:4	Off-white	-ve	-ve	-ve	-ve	-ve
+Polyethylene glycol 400	1:1	Off-white	-ve	-ve	-ve	-ve	-ve
+Diazolidinyl urea (and) Iodopropynyl butylcarbamate,	1:1	Off-white	-ve	-ve	-ve	-ve	-ve
Butyl hydroxy toulene	1:1	Off-white	-ve	-ve	-ve	-ve	-ve
Glycerin	1:1	Off-white	-ve	-ve	-ve	-ve	-ve
Benzyl alcohol	1:1	Off-white	-ve	-ve	-ve	-ve	-ve
Citric acid	1:1	Off-white	-ve	-ve	-ve	-ve	-ve
Mineral oil	1:1	Off-white	-ve	-ve	-ve	-ve	-ve
Water	1:1	Off-white	-ve	-ve	-ve	-ve	-ve

-ve: No change with respect to control

at 40°C/75% RH). There was no change in organoleptic characteristics of the mixture when checked visually (Table 2).

Process Optimization

Product ingredient optimization was done by performing prototype studies, which is summarized in Table 3.

Formulation Characterization

The present study demonstrates that topical nanoemulsion formulated by a cold emulsification process can potentially improve the solubility, bioavailability, and stability of thermolabile drugs, overcoming challenges associated with the hot emulsification process. The zeta potential value of the optimized formulation was –30 mV, ensuring the physical stability of the acyclovir nanoemulsion. The formulation developed in the current research showed vesicle size in the 50 to 70 nm range, indicating better transdermal and trans follicular penetration. The PDI is dimensionless and indicates the particle size distribution in a nanoparticulate system. A very monodisperse system is indicated by PDI values less than 0.05, whereas a very wide particle size distribution is indicated by values more than 0.7 (Table 4).[13,14] A comparative diffusion profile study between nanoemulsion and conventional macroemulsion has shown steady-state flux and better skin penetration of nanoemulsion than the surfactant-based emulsion system.[15] Optimized acyclovir nanoemulsion has also shown significantly better diffusion compared to conventional hot emulsification process based marketed formulation. All the results signify that the cold emulsification process is

Table 3: Optimization of formulation ingredients

Process parameters		*Conventional homogenization*	*Configuration with in-line homogenization*		
		3000 rpm	*4M*	*6F*	*8SF*
Ingredient	%	PB-017	PB-018	PB-019	PB-020
Acyclovir	5.0	5.0	5.0	5.0	5.0
Acrylamide/Sodium acryloyl dimethyl taurate Copolymer/Isohexadecane & Polysorbate 80	4.0	4.0	4.0	4.0	4.0
Benzyl alcohol	2.0	2.0	2.0	2.0	2.0
Butylated hydroxy toluene	0.1	0.1	0.1	0.1	0.1
Glycerine	3.0	3.0	3.0	3.0	3.0
Light liquid paraffin	3.0	3.0	3.0	3.0	3.0
Tween 20	5.0	5.0	5.0	5.0	5.0
Propylene glycol	10.0	10.0	10.0	10.0	10.0
Hydrogenated castor oil	3.0	3.0	3.0	3.0	3.0
Citric acid	0.5	0.5	0.5	0.5	0.5
Purified water Qs (mL)	100.0	100.0	100.0	100.0	100.0
Total process time (minutes)		15	3	5	5

Table 4: Comparative characterization of optimized batches with acivir (marketed formulation)

Formulation	*Assay (%)*	*Impurity of acyclovir -Guanine (%)*	*Zeta potential (mV)*	*Droplet size (nm)*	*Polydispersibility index (PDI)*	*Refractive index (RI)*	*pH*	*Viscosity (cps)*
Acivir	98.20 ± 1.00	0.14 ± 0.02	-2.47 ± 27.15	5770.00 ± 1800.00	0.809 ± 0.032	1.56 ± 0.04	5.05 ± 0.60	90000 ± 3000
PB-017	98.80 ± 0.40^{ns}	0.12 ± 0.01^{ns}	-18.60 ± 3.87^{***}	5800.20 ± 1600.00^{ns}	0.728 ± 0.012^{ns}	1.68 ± 0.05^{ns}	4.72 ± 0.50^{ns}	78000 ± 1000^{**}
PB-018	99.60 ± 0.40^{ns}	0.11± 0.02^{ns}	-27.20 ± 4.54^{***}	244.40 ± 24.00^{**}	0.171 ± 0.010^{***}	1.33 ± 0.05^{**}	4.75 ± 0.36^{ns}	76400 ± 1200_{**}
PB-019	99.90 ± 0.30^{ns}	0.11 ± 0.01^{ns}	-29.40 ± 5.59^{***}	105.10 ± 18.00^{**}	0.299 ± 0.011^{**}	1.33 ± 0.02^{**}	4.71 ± 0.45^{ns}	72000 ± 1500_{***}
PB-020	99.50 ± 0.40^{ns}	0.12 ± 0.01^{ns}	-32.20 ± 5.93^{***}	66.20 ± 10.0^{0*}*	0.251 ± 0.007^{***}	1.32 ± 0.03^{**}	4.73 ± 0.33^{ns}	72000 ± 1400_{***}

Each value is a mean of three determinations ± SD (n = 3)

Table 5: *In-vitro* diffusion profiles of optimized batches compared with acivir (marketed formulation)

Formulation	*Release (%) of acyclovir at various time intervals (time in min)*					
	30	*60*	*120*	*180*	*240*	*360*
Acivir	9.40 ± 1.80	22.30 ± 3.20	45.10 ± 2.50	59.30 ± 1.50	69.60 ± 1.60	70.10 ± 1.00
PB-017	9.20 ± 2.20^{ns}	17.80 ± 2.20^{*}	35.70 ± 1.30^{**}	50.10 ± 1.50^{*}	61.20 ± 2.00^{*}	62.20 ± 2.50^{*}
PB-018	12.30 ± 1.60^{*}	28.70 ± 3.00^{*}	48.90 ± 3.10^{ns}	64.90 ± 3.70^{*}	71.50 ± 3.00^{ns}	71.00 ± 2.20^{ns}
PB-019	13.20 ± 2.50^{**}	31.00 ± 2.00^{**}	50.50 ± 1.20^{**}	67.10 ± 3.80^{**}	76.00 ± 4.00^{**}	76.20 ± 2.00^{*}
PB-020	14.10 ± 0.40^{**}	32.00 ± 1.20^{**}	52.20 ± 2.50^{**}	69.40 ± 3.07^{***}	82.00 ± 4.60^{***}	82.40 ± 4.80^{***}

Each value is a mean of three determinations ± SD (n = 3).

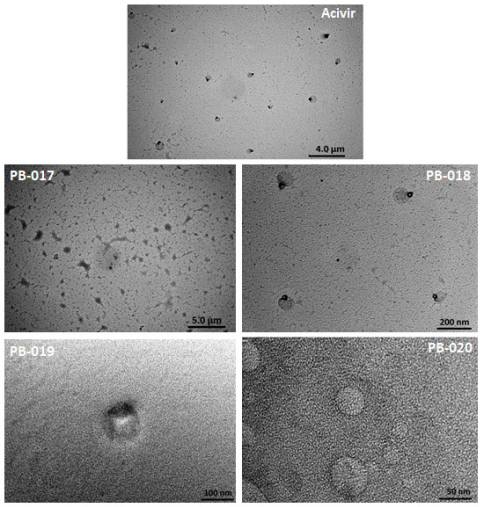

Figure 2: TEM of optimized nanoemulsion formulation batches and Acivir (marketed formulation)

appropriate for developing nanoemulsions of thermolabile and pH-sensitive drugs.

In-vitro Diffusion Kinetics

The release profile of PB-019 and PB-020 nanoemulsions in comparison to Acivir is reported in (Table 5).

TEM micrograph

Figure 2 reports the acyclovir nanoemulsion's TEM micrograph. It demonstrates that the surfaces of nanoparticles are almost smooth and have a spherical form. The distinct and non-aggregated nature of acyclovir-nanoemulsion globules was further shown by TEM examination.

CONCLUSION

This study opens up the avenue for manufacturing a stable, cost-effective, and environment-friendly nanoemulsion of thermolabile drugs as topical products. The cold emulsification technique exhibits notable promise as an efficient approach for formulating topical nanoemulsion of thermolabile drugs.

ACKNOWLEDGMENT

The author expresses her gratitude toward DSKM College of Pharmacy, RKDF University, Bhopal, M.P., India for providing a research environment and all necessary facilities for conducting research.

REFERENCES

1. Hadi AS, Ghareeb MM. Rizatriptan Benzoate Nanoemulsion for Intranasal Drug Delivery: Preparation and Characterization. International Journal of Drug Delivery Technology. 2022;12(2):546-552. DOI: 10.25258/ijddt.12.2.14
2. Nashat BI, Al-Kinani KK. Nanoemulsion Formulation of Leflunomide for Transdermal Delivery: Preparation and Characterization. International Journal of Drug Delivery Technology. 2023;13(1):57-65. DOI: 10.25258/ijddt.13.1.09
3. Ashoor JA, Ghareeb MM. Formulation and *In-vitro* Evaluation of Methotrexate Nanoemulsion using Natural Oil. International Journal of Drug Delivery Technology. 2022;12(2):670-677. DOI: 10.25258/ijddt.12.2.36
4. Brogden RN, Peters DH. Teicoplanin: a reappraisal of its antimicrobial activity, pharmacokinetic properties and therapeutic

efficacy. Drugs. 1994;47(5):823-854. DOI: 10.2165/00003495-199447050-00008
5. Mayson HA, Duaa KZ, Wadiah SB. Anticancer Activity of Mixed Doxorubicin and Pravastatin in Nanoemulsions Against HCT 116 Colon Cancer Cells. International Journal of Drug Delivery Technology. 2017;7(1):63-70. DOI: 10.25258/ijddt.v7i1.8918
6. Nasser ST, Abdulrassol AA, Ghareeb MM. Design, Preparation, and *In-vitro* Evaluation of Novel Ocular Antifungal Nanoemulsion Using Posaconazole as a Model Drug. International Journal of Drug Delivery Technology. 2021;11(3):1058-1064. DOI: 10.25258/ijddt.11.3.71
7. Dahash RA, Rajab NA, Almajidi YQ. Investigation of the Effect of Variable Components on the Preparation and *In-vitro* Evaluation of Lacidipine as an Oral Nanoemulsion Dosage Form. International Journal of Drug Delivery Technology. 2021;11(3):1031-1036. DOI: 10.25258/ijddt.11.3.65
8. Jain S, Shah RP. Drug-excipient compatibility study through a novel vial-in-vial experimental setup: a benchmark study. AAPS PharmSciTech. 2023;124(5):117. DOI: 10.1208/s12249-023-02573-0
9. Tristiana EM, Retnowati, Amalia WF, Widji S. Virgin Coconut Oil as Oil Phase in Tretinoin Nanoemulsion. International Journal of Drug Delivery Technology 2017;7(1):42-44. DOI: 10.25258/ijddt.v7i1.8915
10. Latif MS, Nawaz A, Asmari M, Uddin J, Ullah H, Ahmad S. Formulation development and *in-vitro*/in vivo characterization of methotrexate-loaded nanoemulsion gel formulations for enhanced topical delivery. Gels. 2023;9(3):1-20. DOI: 10.3390/gels9010003
11. Makadia HA., Bhatt AY, Parmar RB, Paun JS, Tank HM. Self-nano emulsifying drug delivery system (SNEDDS): Future aspects. Asian Journal of Pharmaceutical Research. 2013:3:1:21-27. DOI: 10.5958/2231-5691
12. Tristiana EM, Widji S, Noorma R. Skin Penetration of Ubiquinone (Co-Q10) In Nanoemulsion Delivery System Using Virgin Cocunut Oil (Vco). International Journal of Drug Delivery Technology. 2018;8(2):77-79. DOI: 10.25258/ijddt.v8i2.13871
13. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh DF, Javanmard R, Dokhani A, Khorasani S, Mozafari MR. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018:10:2:57. DOI: 10.3390/pharmaceutics10020057.
14. Grace L, Sarala, Valencia W, Shana S, Richard G, Tarun KM. Development and *in-vitro* evaluation of a nanoemulsion for transcutaneous delivery. Drug development and Industrial Pharmacy. 2014;40(3):370-379. DOI: 10.3109/03639045.2012.763137
15. Tiwari G, Singh G, Shekhar R, Tiwari R. Development and qualitative evaluation of periodontal gel containing an antibacterial agent for periodontal disease. Research Journal of Pharmacy and Technology. 2022;15(11):5225-31. DOI: 10.52711/0974-360X.2022.00880