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Formulation and Evaluation of Microemulsion Based in Situ Gel of Acyclovir for Vaginal Delivery

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ABSTRACT

The purpose of the present study was to formulate and evaluate microemulsion based in situ gel of Acyclovir (ACV) for the vaginal delivery. The solubility of ACV in oils and surfactants and co-surfactant was evaluated to identify the components of the microemulsion. Microemulsion region was determined by using the pseudo-ternary phase diagrams for different formulations. Microemulsion formulation was prepared using Labrafil M1994C as oil phase, Cremophor RH40 as surfactant and Polyethylene glycol 400 and Transcutol P as co-surfactant and water. Microemulsion formulations were evaluated for pH, viscosity, conductivity and stability study. In situ gel of ACV, microemulsion was prepared using thermosensitive polymer, poloxamer.In situ gelwas characterized for viscosity, gelling temperature, the effect of dilution on gelling temperature, gelling ability, and in vitro drug release and release kinetics. The globule size of developed microemulsion was less than 100 nm with PDI in the range 0.307 to 0.641. The optimized microemulsion based in situ gel demonstrated shear thinning behaviour, the gelation temperature with and without dilution was in the range of 30-35ºC, and the drug release was sustained over eight hours. Mucoadhesive properties of microemulsion based in situ gel formulations were determined with a texture analyzer using a goat vaginal tissue, and the results indicated that the presence of microemulsion increased the mucoadhesion significantly. Microemulsion based in situ gel was successfully developed for vaginal delivery of Acyclovir.

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INTRODUCTION

Genital herpes is most commonly occurred sexually transmitted infection that is caused by Herpes simplex viruses (Whitley and Roizman, 2001). The virus mainly enters through the skin or mucous membrane, easily bypasses the immune system and may remain latent for many years. Periodic reactivation of the virus leads to recurrent infection, making it difficult to treat.Genital herpes is associated with a high risk of HIV acquisition and considerable morbidity (Looker *et al.*, 2008).

The infection is prevalent in a developed and developing country, and more than 400 million people worldwide are reported to be infected by the virus (Looker *et al.*, 2008). The infection may cause a threat and complications to the reproductive system of the female and hence needs to be effectively treated. The treatment of genital herpes includes the use of antiviral agents like Acyclovir (ACV), Vala-

cyclovir and Famciclovir (Field and Hodge, 2013). These drugs can partially control the symptoms of genital herpes and do not provide the cure. ACV is the most commonly used antiviral agents in HSV management and is a synthetic purine nucleoside analogue that is known to inhibit and inactivate the viral DNA polymerase. The formulations of ACV are available for the systemic and topical purpose, however, due to lower permeation of ACV, being a class III compound, it has limited oral bioavailability (Dias *et al.*, 2002). Due to lower penetration, topical therapy of ACV also offers limited clinical benefits (Field and Hodge, 2013; Kłysik *et al.*, 2018). The drug delivery through mucosa can be facilitated by new drug delivery system based on nanotechnology like microemulsion (ME), nanoparticles and nano vesicular systems (Clercq, 1987; Gide *et al.*, 2013). ME propose significant advantages over other methods including the ability to solubilize polarly as well as nonpolar actives, high solubilization capacity resulting in higher drug loading, thermodynamic stability, small globule size and ease of manufacturing. ME can be further incorporated in gels or creams to retain the formulation in the mucosal cavity (Sahoo and Labhasetwar, 2003).

Vaginal gels, although, are popular conventional formulations, suffers from many limitations including stickiness, nonuniform distribution in the vaginal cavity and possibility of leakage (Fouad *et al.*, 2013). These problems can be addressed by in situ gelling formulations that are liquid at room temperature and transition into a gel at body temperature when administered in the vaginal cavity (Botes, 2012). Poloxamers are thermosensitive polymers that show the solution to gel transition in response to a temperature above a specific concentration.

Few studies are previously reported on ME based gel for ACV delivery to the skin and vaginal mucosa. But there are no reports of ME based in situ gelling systems for vaginal delivery. In the present study, an attempt has been made to formulate and evaluate ACV ME for the vaginal route. This ME was further incorporated into in situ gelling formulations. Were evaluated for globule size, thermodynamic stability, gelling temperature, the effect of dilution on gelling temperature, mucoadhesion and in vitro drug release studies (Ruel-Gariépy and Leroux, 2004; Antimisiaris and Mourtas, 2015).

MATERIALS

Acyclovir was generously gifted by Hetero drug Ltd., Visakhapatnam, India. Poloxamer 407 (Kolliphor P 407) and Poloxamer 188 (Kolliphor P 188) were received from BASF, Mumbai, India. Cremophor RH 40 (Kolliphor RH 40) was obtained from BASF, Mumbai, India. Transcutol P and PEG 400 were purchased from Loba chemicals, Mumbai, India.

METHODS

Solubility study

The solubility of ACV in various oils, surfactants and co-surfactants was determined. An excess amount of ACV was added to 2 ml of oil, surfactant and co-surfactants taken in vials separately. The mixture was vortexed for 10 to 15 min, and then the vials were kept in an orbital shaker at 25 ±0.5°C at 100 rpm for 72 h. The mixtures were centrifuged at 3000 rpm for 15 min to separate the undissolved drug. The supernatant was suitably diluted with methanol, and the concentration of drug was determined by UV spectro photometer (Shimadzu, UV1700, Japan)at 253.0 nm using a previously plotted calibration curve (Choi *et al.*, 1999).

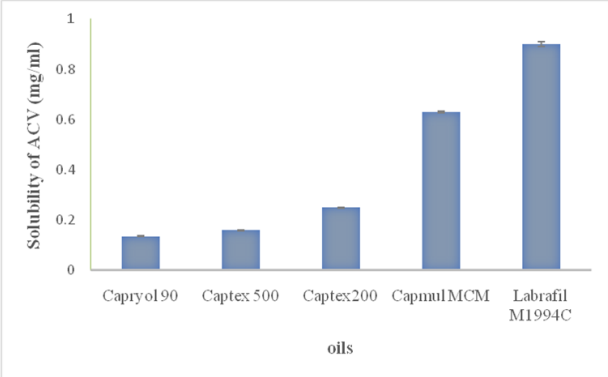

Figure 1: Solubility study of ACV in different oils *Error bars represent standard deviation of three replicates.

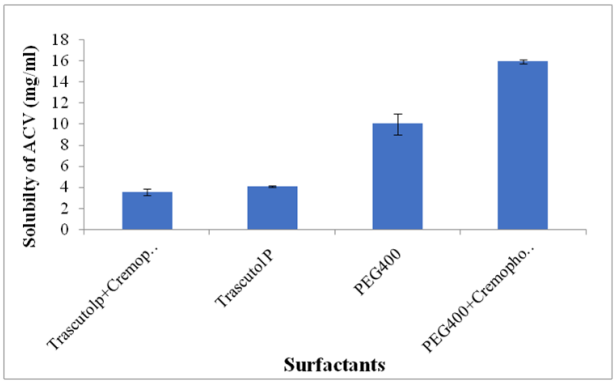

Figure 2: Solubility study of ACV in different surfactants and cosurfactants *Error bars represent standard deviation of three replicates.

Construction of pseudo ternary phase diagram

To identify one phase ME region, pseudo ternary

Table 1: Composition of ACV ME formulation

Batch	ME system	Ratio of S:Cos in Smix	Oil (% w/w)	Smix (%w/w)	Water (%w/w)
M1	LabrafilM1994C+Cremophor RH40+PEG400	1:1	5	25	70
M2	LabrafilM1994C+Cremophor RH40+PEG400	1:2	5	25	70
M3	LabrafilM1994C+Cremophor RH40+PEG400	2:1	5	25	70
M4	LabrafilM1994C+Cremophor RH40+PEG400	1:1	5	15	80
M5	LabrafilM1994C+Cremophor RH40+Transcutol P	1:1	5	25	70
M6	LabrafilM1994C+Cremophor RH40+Transcutol P	1:2	5	25	70
M7	LabrafilM1994C+Cremophor RH40+Transcutol P	2:1	5	25	70

To each 10g of ME formulation 20mg of ACV was added

Table 2: Composition of in situ gelling formulations

Batch	ACV ME M4 (% w/w)	P407(%w/w)	P188 (%w/w)
MEG 1	77	18	5
MEG 2	72	18	10
MEG 3	79	16	5
MEG 4	74	16	10
MEG 5	80	15	5
MEG 6	75	15	10
MEG 7	86	14	0
MEG 8	81	14	5
MEG 9	76	14	10

*Each 10 g of gel contains20 mg of acyclovir

Table 3: Evaluation of microemulsion

Batch	Conductance	Viscosity at 20 rpm (cps)	Percent trans-mittance (%)	Globule size (nm)	PDI	Appearance or clarity before thermo-dynamic stability studies*	Appearance or clarity after thermodynamic stability studies*	
							H/C **	Cent. **
M1	0.312±0.01	90±0.57	86.11±0.0	50.6±0.42	0.406±0.6	+++	++	++
M2	0.264±0.05	82±1.73	92.13±0.5	67.8±0.49	0.231±0.3	+++	++	++
M3	0.180±0.01	80±1.15	78.26±0.9	416.8±0.7	0.641±0.1	+++	+	+
M4	0.112±0.05	94±1.03	87.28±1.8	48.8±0.70	0.307±0.6	+++	+++	+++
M5	0.115±0.00	97±1.15	95.13±0.3	66.0±0.56	0.441±0.9	+++	+++	+++
M6	0.430±0.01	81±0.57	77.32±1.2	466.9±0.4	0.308±0.5	+++	+	+
M7	0.160±0.00	105±0.5	95.79±0.6	129.4±1.2	0.361±0.5	+++	+++	+++

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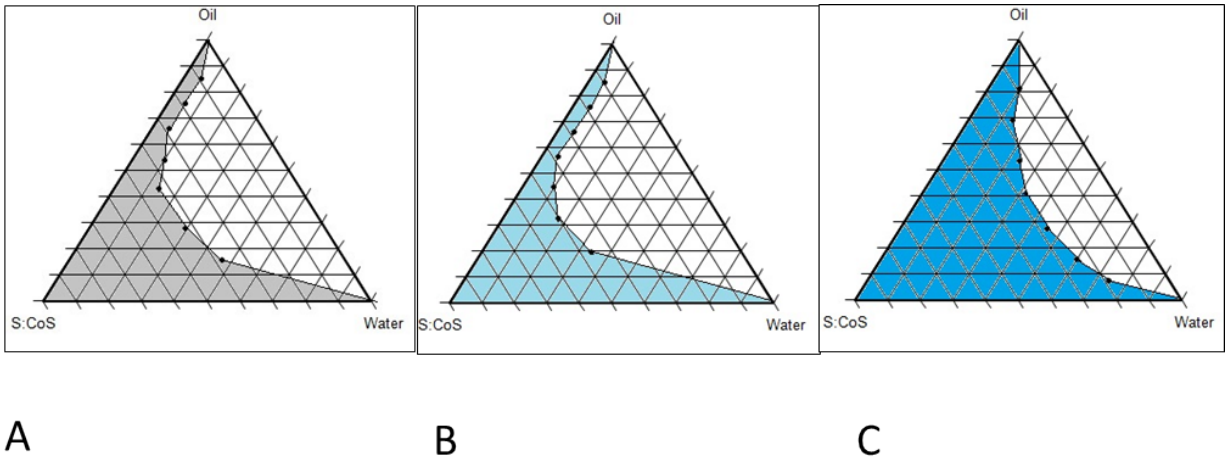

Figure 3: Pseudoternary phase diagram showing microemulsion region of LabrafilM1994C,as oil, water and Smix (Cremophor RH 40: PEG 400) with different ratioof Smix (surfactant: Co-surfactant) A 1:1, B 1:2, C 2:1.

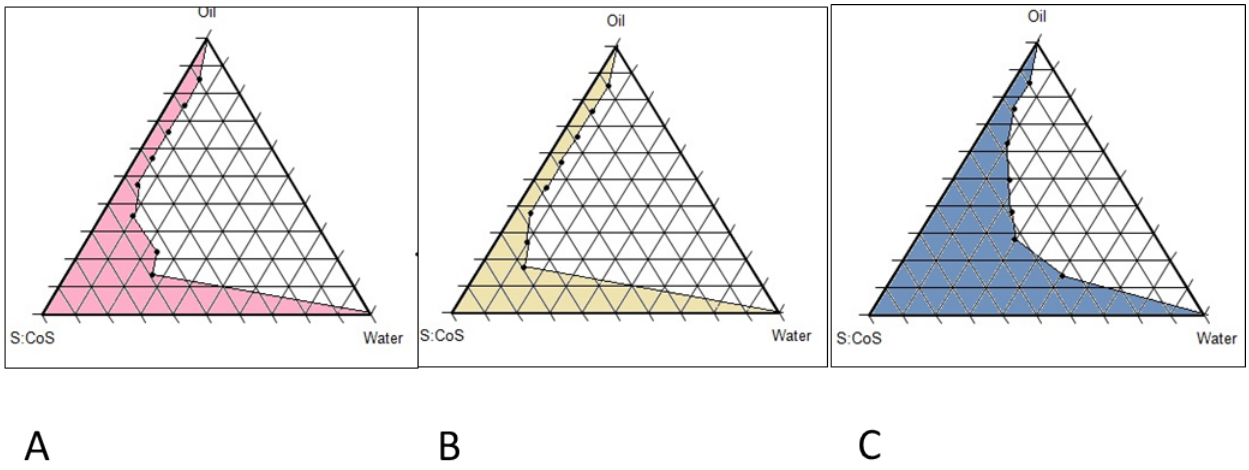

Figure 4: Pseudo-ternary phase diagram showingmicroemulsion region of Labrafil M 1994C asoil, water and Smix (Cremophor RH40: Transcutol P) with different ratio of Smix (surfactant: Co-surfactant) A1:1, B 1:2, C 2:1.

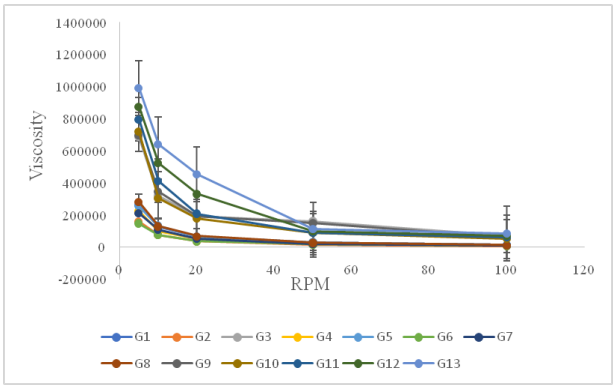

Figure 5: Viscosity of Microemulsion Based In situ Gel *Error bars represent standard deviation of three replicates.

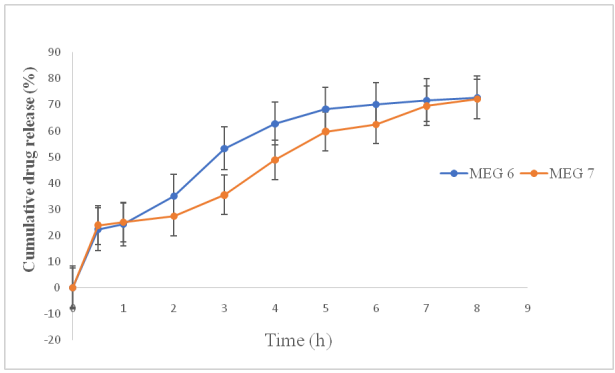

Figure 6: In vitro drug release profile of microemulsion based in situ gel *Error bars represent standard deviation of three replicates.

Table 4: Formulation and evaluation of ME based in situ gelling formulation

Batch	Appearanc	pH	Gelling temp (°C)*	Gelling ability***	Gelling temp after dilution*	Spredabilit (cm)*	Drug content (%)*
MEG 1	+++	3.5	Gel at 4°C	+++	Gel at 4°C	-	96.68±0.11
MEG 2	+++	3.2	Gel at 4°C	+++	Gel at 4°C	-	96.45±0.15
MEG 3	+++	4	17.33±0.7	+++	17.66±1.4	0.1±1.14	95.17±0.34
MEG 4	+++	4.5	21.33±0.71	+++	23.66±0.7	0.6±0.07	95.86±0.33
MEG 5	+++	3.5	26.66±0.71	+++	29.66±0.7	1.8±0.07	95.33±0.18
MEG 6	+++	3.6	32.33±0.70	+++	34.33±1.1	0.5±1.14	97.93±0.15
MEG 7	+++	3.2	31.66±0.69	+++	33.33±0.6	0.8±1.14	96.95±0.43
MEG 8	+++	4.8	35.33±0.71	+	36.66±1.4	2±0.21	97.96±0.35
MEG 9	+++	4.6	40.33±0.69	-	43.66±0.7	2.5±0.15	97±0.13

*Values are expressed as Mean ± SEM(n=3), Grading based on **appearance of ME in situ gel, (+++) clear and transparent,(++) translucent, (+) and ***gelling ability No Phase transition (-), formationof gel after 60 S and collapsed rapidly within 15min (+), formation of gelafter 60 S and gel collapsed within 1 h (++), formation of gel within 60 S and didnot collapse till 6 h.(+++).

Table 5: Data of drug release kinetics

Batch	Zero order	First order	Higuchi matrix		K- Peppas		Hixson Crowell
	R^2	R^2	R^2	k	R^2	N	R^2
MEG 6	0.9340	0.9955	0.9888	32.1191	0.9876	0.5961	0.9894
MEG 7	0.9514	0.9772	0.9713	28.4559	0.9440	0.5083	0.9822

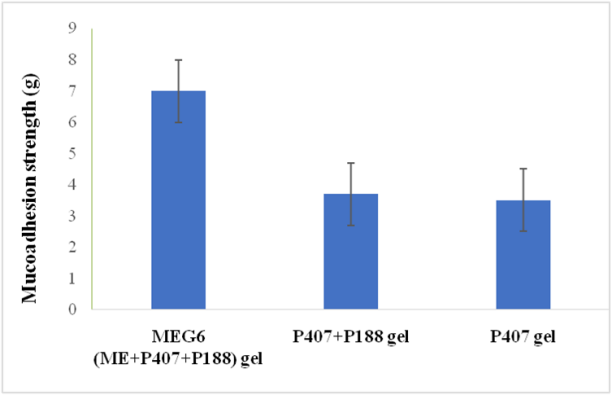

Figure 7: Mucoadhesion strength of based in situgel *Error bars represent standard deviation of three replicates.

phase diagrams of oil, surfactant mixture (surfactant and co-surfactant) and water were plotted. The selected surfactant and co-surfactant were blended (Smix) in ratios, 1:1,2:1, and 1:2. The Smix was mixed with an oil phase to obtain different weight ratios 9:1,8:2,7:3,6:4,5:5,4:6,3:7,2:8 and 1:9 using a magnetic stirrer. The mixtures were slowly titrated with distilled water over the entire phase region. The mixtures were assessed visually for transparency, and the pseudo ternary phase diagrams were constructed using Triplot-software. A monophasic region in the phase diagram was identified as the ME region (Zhu *et al.*, 2009; Khandavilli and Panchagnula, 2007). The pseudo ternary phase diagrams were constructed for system, Labrafil M1994C (oil), Cremophor RH40 (surfactant), PEG400 (as cosurfactant) and Labrafil M1994C (oil), Cremophor RH40 (surfactant), Transcutol P (as cosurfactant).

Formulation of ACV ME

Based on the results of phase diagrams, suitable compositions of ME in the monophasic region of phase diagram were selected. The composition of MEwas selected based on the criteria that the required amount of ACV should get solubilized in the selected ME and the amount of water in ME be higher so that gel formation should be possible. Various ME formulation (Table 1) were prepared by mixing accurately weighted amount of oil, surfactant and co-surfactant in a vial. ACV was dissolved in this mixture with ultra-sonication for 20 min followed by the addition of a fixed amount of water

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with continuous magnetic stirring to obtain clear ME. The ME was further evaluated for pH, conductance, viscosity, per cent transmittance, globule size and zeta potential (Vintiloiu and Leroux, 2008; Mou *et al.*, 2008)

Evaluation of ACV ME

pH

The pH of formulations was determined by pH meter (Toshniwal, CL54, India). The pH meter was calibrated before each use with standard pH 4, 7, and 9 buffer solutions. The pH meter electrode was immersed in the formulation, and the pH was recorded.

Conductance

The conductance of ME formulation was determined by using conductometer. The conductometer was calibrated before each use with standard KCl solutions. The conductometer electrode was immersed in the formulation, and the conductance was recorded (Kantaria *et al.*, 2003; Ghosh *et al.*, 2012).

Viscosity

The viscosity of the prepared ME was determined using Brookfield viscometer (Brookfield, RVDV pro II, USA)with small sample volume adaptor. The Prepared ME solution was transferred in a sample cell which was placed carefully within the adaptor. The guard leg was placed around the adaptor, and the volume of the sample was stirred slowly using motor-driven stirring elements. The viscosity values were recorded at 20 rpm (Mahore *et al.*, 2017; Deshkar *et al.*, 2015).

Per cent transmittance

The UV spectro photometer analyzed the per cent transmittance of the formulation at 600 nm.

Globule size and Zeta potential measurements

Particle size distribution and zeta potential measurement of ME was determined using Particle size analyzer (Horiba, SZ 100, Japan).

Microemulsion sample was directly subjected to particle size and zeta possible measurement without dilutions. The measure was done at 90°angles, and 25°C and the observations were carried out in triplicate.

Thermodynamic stability studies

These studies included exposure of formulation to thermal (both low & high) as well as mechanical stress & observing the effects on the phase separation, clarity of the ME formulation (Moulik *et al.*, 2000; Biais *et al.*, 1982) .The test was carried out in two parts;

Alternate heating (40°C) / cooling (4°C) Cycle

It includes storage of formulations at each of these temperatures viz, 4°C and 40°C alternately for not less than 48h for three cycles. The MEformulations were observed for any kind of instability by evaluating them for any change in phase separation, and optical clarity and only stable formulations were selected for a subsequent test of centrifugation (Yadav *et al.*, 2018).

Centrifugation

It included centrifugation of ME formulation for 30 min at 3000rpm and formulations were observed for instability by evaluating them for change in phase separation and optical clarity.

Formulation of ME based in situ gel

To prepare a ME based in situ gel, the procedure was slightly modified. P407 andP188 were added in a weighed quantity of water and placed at 4◯C for 24 h for complete solubilization of polymer in water. In separate vials, a mixture oil, surfactant and co-surfactant was prepared, to which ACV was dissolved by ultrasonication for 10 min. This mixture was added to poloxamer solutions with continuous stirring to obtain in situ gelling formulations (Table 2) (Ray *et al.*, 1993).

Evaluation of ME based in situ gel

Gelling temperature

The previously cooled (to 4°C) microemulsion based in situ gel formulation (4 g) was placed in a glass vial containing magnetic bar. The solution was heated with continuous stirring on a magnetic stirrer at 200 rpm. The change in the temperature at which the magnetic bar stopped moving, was noted as gelation temperature each measurement was done in triplicate (Zaki *et al.*, 2007; Carla, 2015).

Effect of dilution on gelling temperature

To study the effect of dilution on the gelling temperature of in situ gelling formulations, citrate buffer pH 4.8 was added (0.25 ml per g of gel) to 4 g of formulations in a vial. The gelling temperature of the diluted formulation was determined using a previously described method (Deshkar and Palve, 2019; Cai, 2011).

Gelling ability

The test for gelling ability was conducted using a citrate phosphate buffer pH 4.8. The individual formulation (1 g) was added into 1 ml of Citrate phosphate buffer contained in a glass vial that was kept into water bath incubator at 37 ◯C. The transition of solution to viscous gel was observed visually. Then the time is taken for the collapse of gel, and then the

gelling ability of in situ gel formulation was determined. The formulations were graded as, No Phase transition (-), the formation of the gel after 60 S and collapsed rapidly within 15min (+), the formation of the gel after 60 S and gel collapsed within1 h (++), the formation of gel within 60 S and did not collapse till six h. (+++). Each sample was measured in triplicate (Deshkar and Palve, 2019).

Viscosity

The viscosity of the gel was determined using a Brookfield Viscometer with T-bar spindle. The helipath movement was controlled, and touching of the spindle to any part of the sample holder was avoided. The viscosity values at each rpm like 5, 10, 20, 50, 100 were noted. For the same gel sample, the experiment was repeated thrice, and the average reading was recorded (Andrews, 2009; Iradhati and Jufri, 2017).

Drug content

Microemulsion based in situ gel (1 g) was dissolved in the mixture of methanol and water (1:1). The resultant dispersion was ultrasonicated and shaken for one hour and then filtered. The absorbance of the filtrate was measured at 254.0 nm by UV spectro photometer using methanol and distilled water mixture as blank. The per cent drug content of the gel formulation was calculated using a calibration curve of ACV in the same solvent (Deshkar and Palve, 2019).

Mucoadhesion strength

Mucoadhesive properties ofME based in situ gel formulations were determined using a Texture Analyzer (Brookfield, CT3 Texture Analyzer, USA). A goat vaginal tissue sample was collected immediately after slaughter of the animal and was separated from the underlying tissues. After proper washing with distilled water, the tissue was rapidly frozen (-20°C) and stored in the saline solution. Before testing, vaginal tissue was defrosted at room temperature. The tissue was placed on the base of the texture analyzer. TheME based in situgel formulations (previously equilibrated at 37 $^{\circ}$C) were applied to the aluminium probe of texture analyzer using double-sided adhesive tape. The vaginal tissue was moistened with citrate buffer pH 4.8. The probe of texture analyzer was lowered to make contact with the tissue. After establishing the contact for 20 S with contact load of 10 g, the probe was withdrawn at a rate of 1 mms^{-1}. The force required to detach the gels from goat vaginal tissue was measured asmucoadhesive strength (g). The readings were taken in triplicate on the same vaginal mucosa (Deshkar and Palve, 2019; Han *et al.*, 2006).

In vitro drug release and release kinetics

The in vitro drug release studies were carried out in citrate phosphate buffer pH 4.8 using a dialysis bag in modified USP dissolution apparatus I. a precise amount (3g) of ME based in situ gelling formulation was placed inside the dialysis bag which was previously soaked overnight in citrate phosphate buffer pH 4.8 medium. Both the ends of the dialysis bag were tied to the basket rod of dissolution assembly. The bags were immersed in the 100 ml of dissolution medium (citrate phosphate buffer pH 4.8) at 37°C with a stirringspeed of 50 rpm. With an hourly interval, the dissolution medium was withdrawn, and the UV spectro photometer analyzed the drug release at 254.0 nm. The same volume of dissolution medium was replaced in the flask to maintain the sink condition. The drug release was expressed as the average of three experiments. To determine the mechanism of drug release, the data of drug release was fitted into different kinetic models, viz. zero order, first order, Higuchi matrix, Korsemayer-Peppas (Ray *et al.*, 1993; Salih *et al.*, 2018).

RESULTS AND DISCUSSION

Solubility study

The microemulsion, consisting of oil, surfactant, cosurfactant,drug and water should be clear and monophasic liquid at ambient temperature. Solubility studies were aimed at identifying suitable oil, surfactant and cosolvent system for the development of Acyclovir microemulsion. Determining the appropriate oil, surfactant and cosurfactant having maximum solubilizing potential for the drug under investigation is very important to achieve optimum drug loading. The results of Acyclovir solubility in various oils, surfactants and co-surfactants are shown in Figures 1 and 2. ACVhas considerably low solubility in oils. This might be because of intermediate solubility of this drug candidate in both water and lipids with a log P value of -1.56.

Among the various oils screened, ACV demonstrated highest solubility in Labrafil M1994C (0.91$\pm$0.01 mg/ml) followed by Capmul MCM (0.629 $\pm$0.002mg/ml). The solubility of ACVwas found to be higher in cosolvents, Polyethylene glycol 400 (10.0$\pm$1 mg/ml) and Transcutol P (4.05$\pm$0.07mg/ml).

The solubility in the surfactant-cosurfactant blend (1:1 ratio) was determined and was observed higher for Cremophor RH 40 and Polyethylene glycol blend(15.98$\pm$0.16mg/ml) as compared to Cremophor RH40 and Transcutol P (3.49$\pm$0.28mg/ml) blend. Considering, the results of solubility study,

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Labrafil M1994C was selected as oil, Cremophor RH 40 as a surfactant and both, Transcutol P and Polyethylene glycol as cosurfactants.

Construction of Pseudo Ternary Phase Diagrams

Figures 3 and 4 indicate pseudo ternary phase diagrams for system, Labrafil M1994C (oil), Cremophor RH40 (surfactant), PEG400 (as cosurfactant) and Labrafil M1994C (oil), Cremophor RH40 (surfactant), Transcutol P (as cosurfactant) respectively. The shaded area in the phase diagram indicates one phase microemulsion region.

The figure indicates higher microemulsion region with higher surfactant proportion in the Smix (S: Cos ratio, 2:1) when compared to S: Cos ratio 1:1 and 1:2. Type of co-surfactant also had a significant impact on one phase region. Phase diagram with polyethene glycol demonstrated higher microemulsion region that with transcutol P.

Formulation of ACVME

From the pseudo ternary phase diagrams, a series of microemulsion formulations with varying concentrations of oil, surfactant, co-surfactant and water were selected. Following points were considered while selecting the various compositions for microemulsion.

A. Dose of drug

As ACV has limited solubility in both oil and water, the amount of drug loading in ME was a significant concern. The compositions were selected from the ternary phase diagram in such a way that a minimum of 20 mg of Acyclovir should be solubilized in 10 g of ME.

B. The droplet size of ME

ME has globule size below 100 nm and are thermo dynamically stable. The formulations that were stable during freeze-thaw cycles and had globule size below 100 nm were considered for further study.

C. The amount of external phase

As ME was further incorporated into in situ gelling formulations, the proportion of water in the formulation was very crucial. For sol to gel transition of poloxamer, a ratio of water should be higher than oil, surfactants and cosurfactants. The formulations containing 70 % or more water were selected for ME.

Evaluation of ME

The pH of ME was found to be in the range of 4.2 to 4.8. The pH was found to be suitable for the acidic pH of the vagina. The conductance of microemulsion formulations was found in the range of 0.112 to 0.430 micro Siemens/cm. Addition of the additional amount of water phase into the formulation had no adverse effects on system stability. In an unstable emulsion system, as phase separation occurs, the conductivity values are significantly reduced. The viscosity of ME formulations was found to be dependent on the proportion of surfactant and co-surfactant proportion. The droplet size of microemulsion formulation is a critical parameter in enhancing drug permeation through the skin. The smaller the droplet size, the larger will be the interfacial surface area provided for drug permeation.The mean droplet size of the selected microemulsion formulations is reported in Table 3 . The droplet size of the ME formulations, M3, M6 and M7 were larger than 100 nm, and the formulations were not transparent, the same is evident from the lower transmittance values. The other ME formulations werewith globule size less than 100 nm and appeared clear and transparent. The globule size was found to be dependent on the type of co-surfactant. In the case of Transcutol P as co-surfactant, a higher amount of co-surfactant than surfactant (ratio 1:2) resulted in larger globule size. However, in the case of polyethene glycol 400, with an increase in the amount of co-surfactant in proportion to surfactant (1:2) resulted in smaller globule size. This could be attributed to lower viscosity of PEG than transcutol resulting in reduced globule size. The decrease in the droplet size reflects the formation of a better closed packed film of the surfactant at the oil/water interface, thereby stabilizing the oil droplets. The microemulsions with S: Cos ratio of 1:1 demonstrated the smallest globule size and were thermodynamically stable.

The ME formulations were subjected to different thermodynamic stability testing by using the heating-cooling cycle and centrifugation cycle to evaluate the possibility of metastable formulations. The results of thermodynamic stability studies (Table 3) revealed that there was no significant change in the microemulsion formulations, M4, M5 and M7. No phase separation, turbidity and creaming or cracking was observed. No signs of precipitation of drug, in the formulation were seen. Thus, these formulations were found to be thermo dynamically stable. The formulations M1, M2, M3 and M6 were found to be unstable and became turbid after stress testing. These formulations were not considered for further study. The zeta potential was evaluated for M4 formulation and observed as -4.1mV. Viewing the results of thermodynamic stability and globule size and polydispersity index values formulation M4 was selected for the formulation of in situ gelling formulations. The polydispersity index of 0.307 indicates less wide size distribution of glob-

ule.

Appearance and pH

Table 4 indicates the evaluation of in situ gelling formulations. All the formulations were clear and transparent, and the pH of the formulation was in the range of 3.2 to 4.8.

Gelation temperature and effect of dilution on gelling temperature

The concentration of P407 and P188 plays a vital role in sol to gel transition of in situ gelling formulations. Poloxamer is a thermoreversible polymer with PEO (Polyethyleneoxide) and PPO (Polypropyleneoxide) blocks. At higher temperature, the relatively hydrophobic PPO blocks are desolvated, resulting in the formulation of micelles. At higher poloxamer concentration, the number of micelles increases that lead to tight packing and transition to gel form. The literature and some of our previous studies indicated a stable in situ gelling formulation at 18% w/w concentration of P407 (Biais *et al.*, 1982). Therefore, in situ gelling formulations with 18% P407 and 18% P407 with 5% P188 were prepared. However, these formulations formed a gel structure even at 4ºC. This could be due to the presence of microemulsion droplets in the micelle core resulting in swollen micelles, which immobilized the water—this indicated need to reduce the poloxamer concentration. Various formulations were prepared by reducing the concentration of P407 to 16%, 15% and 14% w/w. As P407 concentration was decreased, the gelling temperature was increased. Addition of P188 to P407 increased the gelation temperature of formulations. In P188, the ratio of PEO: PPO is higher (8:2) than P407 (7:2) due to to which the gelation temperature increases. For formulation MEG 9, due to low P407 concentration (14% w/w), the gel formation was not observed at 37ºC as the gelling temperature was 40ºC. A vaginal in situ gelling formulation should have gelling temperature in the range of 30-35ºC, as low gelling temperature results in difficulty in handling & higher gelling temperature may cause problems of leakage through vaginal cavity due to improper gel formation. Formulations MEG 6, MEG 7 and MEG 8 demonstrated gelation temperature in the desired range.

As gel may undergo dilution in the vaginal cavity, the effect of dilution on the gelling temperature of in situ gelling formulations was also studied. With dilution, the increase in the gelling temperature was observed. For MEG 8 formulation, the gelation temperature after dilution was increased to 36ºC whereas, for MEG6 and MEG7, it was in the range of 30-35ºC after dilution. Therefore, formulations MEG 6 with 15% P407 and 10% P188 and MEG7 with 14% P407 were considered for further study.

Viscosity

All the preformed gel at 37ºC exhibited shear thinning behaviour. Increase in the poloxamer concentration significantly increased the viscosity of the gel. Figure 5 presents viscosity of ME based in situ gel.

Viscosity of Microemulsion Based In situ gel

In vitro drug release

Figures 5 and 6 presented in vitro drug release profile of ME based in situ gel. The drug release was found to be sustained over 8h for ME based in situ gel formulation. There was no significant difference in the drug release of MEG 6 and MEG 7 formulation.

A burst release was observed during the first 30 min of dissolution. This could be due to the presence of a drug in the external phase of ME. The drug release kinetics data is presented in Table 5 . The n value ofKorsemayer-Peppas model was observed as 0.59 and 0.5 that indicates a drug release mainly by fickiandiffusion process.

Ex-vivo mucoadhesion

The mucoadhesion determines the residence time of the gel formulation. The self-cleaning action of vaginal can affect the residence time of the formulation. Therefore, ex-vivo mucoadhesion is an important parameter.The mucoadhesion of MEG 6 formulation (containing ME with 15% P407 and 10% P188), formulation without microemulsion (containing 15% P407 and 10% P188) and formulation with only P407 (15% P407) are present in Figure 7.

Formulation with only P407 and with P407 and P188 revealed no significant difference in mucoadhesion, whereas addition of ME increased the mucoadhesion to a greater extent. The small size of ME globulesresulted in close contact with the goat mucosa resulting in higher mucoadhesion.

CONCLUSIONS

The ME formulation of ACV was developed using Labrafil M1994C, Cremophor RH 40, and PEG400. The ME was thermodynamically stable, with globule size of 50 to 100 nm. The ME formulation was further incorporated intoin situ gel using P407 and P188. The optimized ME based in situ gel exhibited shear thinning behaviour, the gelation temperature with and without dilution was in the range of 30-35ºC and demonstrated sustained drug release over 8h. Conclusively, a ME based in situ gel, was successfully developed considering the suitability to the vaginal cavity.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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