Original article

Formulation and Evaluation of Topical Acyclovir Gel Using Different Polymers

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Abstract

Background: Acyclovir is usually given as protracted therapy and at greater dosing frequency for complete remittance of the disease due to its low oral bioavailability, but this is associated with side effects. Topical preparations of acyclovir are the alternative route of administration to treat local dermatological diseases caused by herpes simplex virus.

Objective: To prepare and evaluate the topical gel formulations of acyclovir by using different polymers.

Materials and Methods: Acyclovir gels were formulated by using three types of polymers, namely carboxymethylcellulose sodium (CMC Na), hydroxypropyl methylcellulose (HPMC) and carbopol-940 as gelling agents at different concentrations. They were evaluated for several physicochemical characteristics including physical appearance, grittiness, viscosity, spreadability, pH, drug content uniformity and *in vitro* drug release studies. The *in vitro* drug release of acyclovir from the selected gel formulations was evaluated as per the procedure described in United States Pharmacopoeia (USP), by using the standard 40

mesh stainless steel dissolution basket (USP Apparatus 1) containing cellulose acetate membrane with phosphate buffer pH 6.8 as the dissolution medium.

Results: Among all prepared gel formulations, formulation F8 containing 3% *w/w* of carboxymethylcellulose sodium was selected as optimal gelling agent in acyclovir gel formulation due to its desired physicochemical characteristics and it showed the highest acyclovir *in vitro* release rate of 96.21 ± 0.92% over 5 hours.

Conclusion: The release of acyclovir from the gel formulations was significantly affected by the type and concentration of polymer (*p*-value < 0.05).

Keywords: Acyclovir, carbopol-940, carboxymethylcellulose sodium, hydroxypropyl methylcellulose, *in vitro* drug release studies.

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Introduction

Acyclovir is an antiviral medicine primarily used to treat herpes simplex virus (HSV) infections, varicella-zoster and herpes zoster.[1-2] Acyclovir has relatively low oral bioavailability (15% to 30%) mainly due to poor water solubility and its absorption is variable, slow and incomplete.[3] Therefore, acyclovir

is usually given as protracted therapy and at greater dosing frequency for complete remittance of the disease. However, this may lead to unintended side effects for some people.

Topical preparations of acyclovir are the alternative route of administration for overcoming the challenge of poor bioavailability to treat dermatologic manifestations of herpes simplex. Drug delivery across the skin enables drugs to be targeted locally to the desired delivery area, hence, potential systemic side effects can be significantly reduced. Furthermore, it avoids metabolic degradation, gastrointestinal irritation, first-pass metabolism and frequent dosing associated with oral therapy of acyclovir.[4,5] Several semisolid preparations are used for topical drug deliver across the skin, and one of this is "gel".

The essential part of a gelling system is made up of a variety of polymers. Gel formulations show variation with the variability of polymer type and concentration which affect drug release and hence the formula quality which must be optimised. Among the many reasons for this study to be conducted is that there is no similar research study done for acyclovir gel formulation by using different types of polymers at different concentrations. Therefore, the main objectives of this research study include:

1. To formulate topical acyclovir gel formulations by using three types of polymers as gelling agents with different concentrations, namely carboxymethylcellulose sodium (CMC Na), hydroxypropyl methylcellulose (HPMC) and carbopol-940.

2. To evaluate the physicochemical characteristics of the formulated gels.
3. To study the effect of polymer type and concentration on the *in vitro* release rate of acyclovir from the prepared gel formulations.

Materials and Methods

Materials

Acyclovir (gift sample from Hovid research laboratory), carbopol-940, hydroxypropyl methylcellulose, M.N. 86,000, viscosity 4,000 cPs (2% solution), carboxymethylcellulose sodium (medium viscosity), 1,2 - propylene glycol, methylparaben, triethanolamine, sodium hydroxide and potassium dihydrogen phosphate.

Methods

Preparation of gels

In semisolid dosage form, gelling agents are used at a concentration of 0.5% to 10%, depending on the agent.[6] In this study, acyclovir gels were prepared by using three different polymers, namely CMC Na, HPMC and carbopol-940 at different concentrations of each polymer (1%, 3% and 5% *w/w*). The concentration of acyclovir in all the formulations remained constant (1% *w/w*). The composition of different formulations is listed in Table 1.

Table 1: Composition of acyclovir topical gel formulations (% *w/w*).

Ingredients	Formulation code								
	F1 (g)	F2 (g)	F3 (g)	F4 (g)	F5 (g)	F6 (g)	F7 (g)	F8 (g)	F9 (g)
Acyclovir	1	1	1	1	1	1	1	1	1
Carbopol-940	1	3	5	-	-	-	-	-	-
HPMC	-	-	-	1	3	5	-	-	-
CMC Na	-	-	-	-	-	-	1	3	5
Propylene glycol	20	20	20	20	20	20	20	20	20
Methylparaben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine	1.0	1.0	1.0	-	-	-	-	-	-
Distilled water q.s.	100	100	100	100	100	100	100	100	100

The gel bases were prepared by dispersing the required amount of the polymers in 10 g of propylene glycol (PG) and 50 g of distilled water with constant stirring by using a spatula at 75°C. Adequate amount of triethanolamine was added to adjust the pH of carbopol-940 gels. Then, the gel bases were left overnight at room temperature (25 ± 2°C) to gain complete hydration and swelling of the polymers.

On the other hand, accurately weighed 1 g of acyclovir powder and 0.1 g of methylparaben were dissolved in 10 g of PG and the remaining amount of distilled water to make up the final volume with constant stirring by using a magnetic stirrer (350 rpm for 30 minutes at 75°C). Next, the solvent blends were transferred slowly to the previously formed CMC Na, HPMC and carbopol-940 gel bases. Lastly, the mixtures were continuously stirred

gently with a spatula for an additional 30 minutes to obtain homogenous gels.

Evaluation of prepared gel formulations

Physical appearance: The prepared gels were observed visually from the container for colour, homogeneity, transparency, and consistency.[6]

Grittiness: The prepared gels were assessed microscopically for the absence of particles.[6]

pH measurements: Eutech CyberScan pH 510 meter was used to measure the pH of the prepared gels.[7] The pH meter was calibrated with standard buffer solutions at a pH of 4.00, 7.00 and 10.00 before starting pH measurement. After calibration, the electrode of the pH meter was immersed into the sample 2 minutes prior to taking the pH reading at room temperature. Measurements were conducted in triplicate for each prepared gel formulation.

Viscosity studies: The viscosity of the prepared gels was measured by using a Brookfield viscometer at room temperature.[7] Spindle number 07 was selected, and it was lowered perpendicularly into the gel. Later, the spindle was rotated at 10 rpm until the readings were stabilised and the corresponding dial reading was recorded in centipoises (cPs).

Spreadability: The spreadability of the prepared gels was measured by spreading 1 g of each gel formulation on a 2 cm original diameter circle pre-marked on a glass plate. This glass plate was covered with a second glass plate of the same

dimension. Later, an object weighs 1000 g was placed on top of the two slides for 1 minute and the diameter of the circle was measured (in cm) after spreading of the gel.[8,9] Measurements were conducted in triplicate for each prepared gel formulation. Spreadability was calculated by using the following formula:

Spreadability = X_1 cm – 2 cm. where, X_1 = the spreading diameter

Drug content uniformity

One gram of accurately weighed gel formulation (equivalent to 10 mg of acyclovir) was dissolved in 100 mL of phosphate buffer pH 6.8 with constant stirring by using a magnetic stirrer at 350 rpm for 1 hour to get complete solubility of the drug. From that 10 mL of solution was taken and diluted to 100 mL with the same buffer solution. After that, the absorbance readings were measured by using Beckman Coulter DU®730 Ultraviolet-Visible (UV-Vis) spectrophotometer at 250 nm. Then, the amount of acyclovir (µg/mL) was determined from the standard calibration curve.[7] Measurements were conducted in sextuplicate for each prepared gel formulation. The percentage of drug content in different formulations was calculated by using the following formula:

$$\text{Percentage of Drug Content} = \frac{\text{Concentration (µg/mL) x Final Volume x Dilution Factor / 1000}}{\text{10 mg}} \text{ X } 100\%$$

where, Final Volume = 100 mL, Dilution Factor = $\frac{\text{100 mL}}{\text{10 mL}} = 10$

In vitro drug release

The *in vitro* release of acyclovir from the selected gel formulations was evaluated as per the procedure described in United States Pharmacopoeia (USP), by using the standard 40 mesh stainless steel dissolution basket (USP Apparatus 1).[10] Phosphate buffer pH 6.8 was used as the dissolution medium throughout the test. The basket screen was covered with cellulose acetate membrane. The membrane was soaked overnight in the dissolution medium prior to use. The bath temperature was kept constant at 32 ± 0.5°C throughout the test to reflect normal skin temperature. Accurately weighed 5 g of each selected gel formulations (equivalent to 50 mg of acyclovir) were filled into the reservoir of the basket apparatus. The appropriate amount of the dissolution medium (900 mL) was added to the dissolution vessel and the shafts were rotated at 200 rpm to start the test. Aliquots of 10 mL were withdrawn at time intervals of 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 minutes. They were replaced immediately by same amount of fresh dissolution medium to maintain constant volume. After that, the amount of acyclovir in the samples was analysed by measuring the absorbance readings using Beckman Coulter DU®730 UV-Vis spectrophotometer at 250 nm and the cumulative percentage drug release of each selected formulation was calculated.

Analytical method validation

Determination of the wavelength of maximum absorbance

Accurately weighed 0.1 g acyclovir powder was dissolved in adequate amount of phosphate buffer pH 6.8 and the volume was

finally made up to 100 mL with the same buffer solution in a volumetric flask to get a standard stock solution (1 mg/mL). After that, 1 mL of standard stock solution was withdrawn and diluted to 100 mL with the same buffer solution in another volumetric flask to obtain a working standard solution (10 µg/mL). Later, the working standard solution was scanned from 200 to 700 nm on a Beckman Coulter DU®730 UV-Vis spectrophotometer.[11]

Robustness

The robustness of the proposed UV-Vis spectrophotometric method was tested by analysing the acyclovir working standard solution under different wavelength conditions (250 nm ± 1). The absorbance readings were determined in sextuplicate for each wavelength condition and the results were represented as relative standard deviation (RSD).

Linearity and range

The establishment of linearity requires a minimum of five concentrations of the target analyte.[12] One millilitre of prepared standard stock solution was withdrawn and the volume was finally made up to 100 mL with phosphate buffer pH 6.8 in a volumetric flask to get a concentration of 10 µg/mL and serial dilutions for linearity were prepared to obtain required concentrations of 5, 2.5, 1.25 and 0.625 µg/mL. Next, the absorbance of the diluted sample solutions was determined in triplicate at 250 nm by using Beckman Coulter DU®730 UV-Vis spectrophotometer at a concentration range of 0.625-10 µg/mL.

A calibration curve of absorbance versus concentration (µg/mL) was plotted.

Specificity

The specificity of the proposed UV-Vis spectrophotometric method was evaluated by comparing the ultraviolet (UV) spectra of blank gels (placebo) against the acyclovir working standard solution. Moreover, the selected acyclovir gel formulations (sample) were scanned from 200 to 700 nm on a Beckman Coulter DU®730 UV-Vis spectrophotometer and checked for any changes in the UV spectra.

Accuracy

The accuracy of the proposed UV-Vis spectrophotometric method was evaluated with the help of the percentage of recovery, SD and RSD by using recovery experiments.[12] Samples were prepared at three levels 80%, 100% and 120% of the test concentration (10 µg/mL*)* by using the prepared standard stock solution. Next, the absorbance of each level was taken in triplicate by using Beckman Coulter DU®730 UV-Vis spectrophotometer at 250 nm.[13]

Precision

The precision of the analytical method was demonstrated by intra-day precision (repeatability) and inter-day precision (intermediate precision).[12] The intra-day precision was tested by performing the *in vitro* drug release studies on six determinations of the similar formulation in the same day, whereas the inter-day precision was

evaluated by performing the *in vitro* drug release studies on six determinations of the similar formulation per day for three consecutive days.[12] The mean, SD and RSD for each of the selected formulations were calculated from the observed absorbance readings by using the final time point of the *in vitro* drug release studies at 300 minutes.

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to perform statistical comparisons by using IBM SPSS Statistic version 21.0 software at a significance level of p-value < 0.05.

Results

Evaluation of prepared gel formulations

Physical appearance

Table 2: Physicochemical characteristics of the formulated gels.

Formulation Code	Homogeneity	Colour	Consistency	Transparency	Grittiness	pH	Viscosity (cPs)	Spreadability (cm)	Drug Content (%)
F1	+++	White	Semi solid	Translucent	No	6.31 ± 0.02	21850	3.93 ± 0.06	99.1 ± 0.24
F2	+++	White	Semi solid	Translucent	No	5.22 ± 0.01	32950	3.60 ± 0.10	97.7 ± 0.23

F3	++	White	Semi solid	Translucent	No	4.63 ± 0.03	34200	2.83 ± 0.15	93.1 ± 0.22
F4	+++	White	Semi fluid	Transparent	No	5.11 ± 0.02	200	7.13 ± 0.21	95.1 ± 0.24
F5	+++	White	Semi solid	Translucent	No	5.04 ± 0.02	2450	5.63 ± 0.15	93.8 ± 0.44
F6	++	White	Semi solid	Translucent	No	5.02 ± 0.02	45300	3.97 ± 0.15	90.7 ± 0.46
F7	+++	White	Semi fluid	Transparent	No	6.14 ± 0.02	350	7.83 ± 0.15	93.5 ± 0.31
F8	+++	White	Semi solid	Translucent	No	5.98 ± 0.02	5050	5.27 ± 0.12	96.0 ± 0.42
F9	++	White	Semi solid	Translucent	No	6.38 ± 0.01	30750	3.43 ± 0.15	91.7 ± 0.21

Notes:

Excellent +++; Good ++; Poor +

Semisolid = More viscous; Semifluid = Less viscous

***In vitro* drug release studies (Table 3)**

Table 3: Cumulative percentage drug release of formulations F2, F5, F8 and F9 at different time intervals.

Time (minutes)	***Cumulative Drug Release (%)**			
	Carbopol-940 3% gel (Formulation F2)	HPMC 3% gel (Formulation F5)	CMC Na 3% gel (Formulation F8)	CMC Na 5% gel (Formulation F9)

0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
30	15.29 ± 0.43	18.55 ± 0.13	23.28 ± 0.31	9.64 ± 1.48
60	21.60 ± 1.46	29.02 ± 1.62	35.78 ± 4.26	16.29 ± 2.05
90	27.89 ± 3.48	40.38 ± 2.29	45.93 ± 4.42	23.39 ± 2.01
120	34.85 ± 6.72	48.00 ± 0.71	57.15 ± 8.24	30.71 ± 3.45
150	41.86 ± 4.78	54.11 ± 0.21	64.24 ± 7.80	36.14 ± 3.38
180	48.63 ± 3.73	60.93 ± 0.58	74.03 ± 6.45	40.51 ± 2.56
210	55.29 ± 2.37	69.64 ± 0.88	81.88 ± 6.38	44.68 ± 2.87
240	61.29 ± 1.96	77.42 ± 0.61	89.37 ± 5.78	48.40 ± 2.53
270	67.48 ± 1.90	85.86 ± 2.06	95.21 ± 0.90	52.82 ± 3.00
300	72.61 ± 2.39	93.57 ± 0.77	96.21 ± 0.92	57.00 ± 3.07

*The results were expressed as mean ± SD; n=18.

Analytical method validation (Table 4-5)

Table 4: Results of robustness studies at three different wavelengths.

	Wavelength (nm)		
	249	250	251
*Mean absorbance ± SD	0.554 ± 0.001	0.558 ± 0.001	0.559 ± 0.001
RSD (%)	0.18	0.18	0.18

*n=6; RSD = relative standard deviation.

Table 5: Results of accuracy studies.

Recovery Level	Concentration (µg/mL)	Concentration Found	% Recovery	Mean % Recovery	SD	RSD (%)
80%	8	7.914	98.93	99.27	0.310	0.31
	8	7.946	99.33			
	8	7.963	99.54			
100%	10	9.933	99.33	99.33	0.325	0.33
	10	9.966	99.66			
	10	9.901	99.01			
120%	12	11.936	99.47	99.43	0.476	0.48
	12	11.985	99.88			
	12	11.871	98.93			

Precision (Table 6-7)

Table 6: Results of intra-day precision studies.

Formulation Code	*Mean absorbance	SD	RSD (%)
F2	2.238	0.043	1.92
F5	2.989	0.056	1.87
F8	3.078	0.000	0.00
F9	1.892	0.035	1.85

*n=6.

Table 7: Results of inter-day precision studies.

Formulation Code	*RSD (%)			Mean RSD (%)
	Day 1	Day 2	Day 3	
F2	1.92	2.06	1.99	1.99
F5	1.87	1.95	2.01	1.94
F8	0.00	0.00	0.00	0.00
F9	2.04	1.99	1.85	1.96

*n=6 (six determinations per day for three consecutive days).

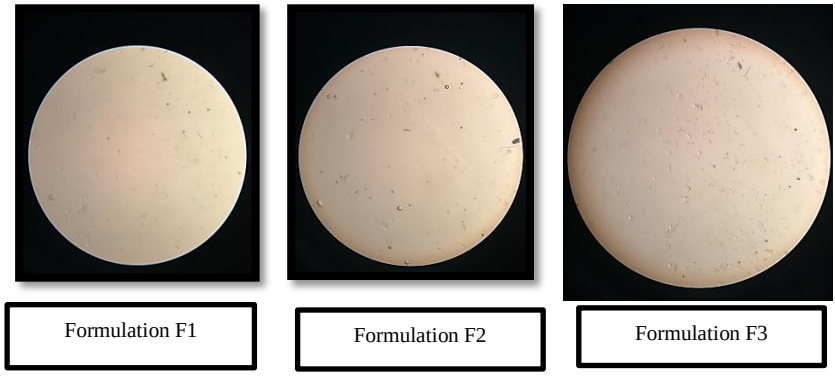


Figure 1: Light microscopy images of acyclovir gels (40x magnification) formulated by using carbopol-940 at (a) 1% *w/w*, (b) 3% *w/w* and (c) 5% *w/w*.

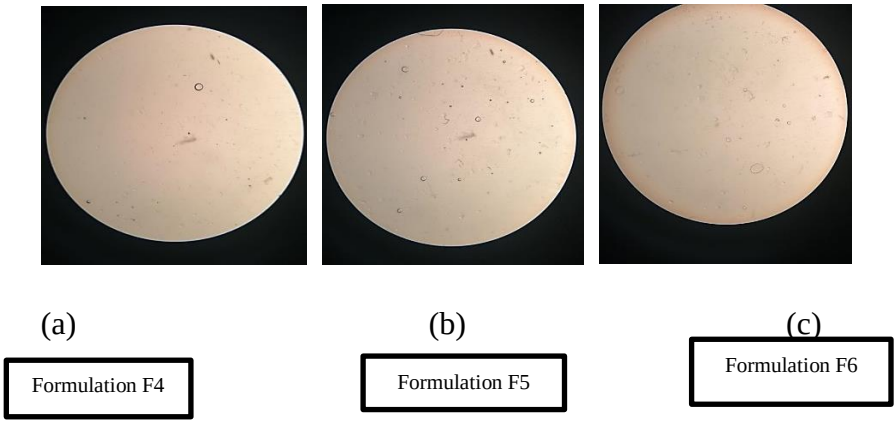
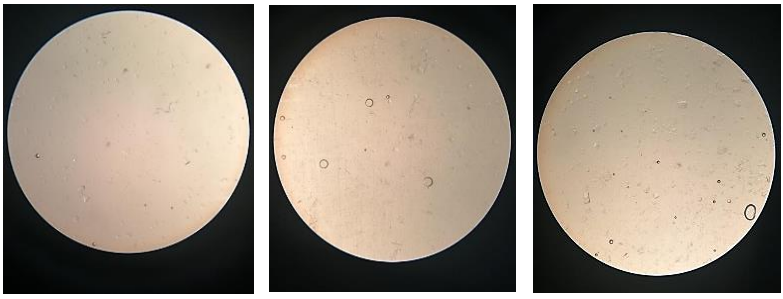


Figure 2: Light microscopy images of acyclovir gels (40x magnification) formulated by using HPMC at (a) 1% *w/w*, (b) 3% *w/w* and (c) 5% *w/w*.



(a) (b) (c)

Formulation F7 Formulation F8 Formulation F9

Figure 3: Light microscopy images of acyclovir gels (40x magnification) formulated by using CMC Na

Discussions

Evaluation of prepared gel formulations

Physical appearance

a) **Homogeneity (Table 2):** All formulated gels were evaluated for the existence of any aggregates and their appearance. Formulations F1, F2, F4, F5, F7 and F8 were smooth and showed excellent homogeneity with the absence of lumps, whereas formulations F3, F6 and F9 were stiff and showed good homogeneity with little presence of lumps. This can be attributed to the concentration of polymer was highest in formulations F3, F6 and F9 at 5% *w/w* when compared to other formulations.
b) **Colour (Table 2):** All formulated gels were white in colour.
c) **Consistency (Table 2):** All formulated gels were semisolid dosage form except formulations F4 and F7 which were semifluid dosage form. This can be attributed to the

concentration of HPMC and CMC Na were lowest in formulations F4 and F7 respectively at 1% *w/w* when compared to other formulations. The factor was not being applied to formulation F1 containing 1% *w/w* of carbopol-940 because this type of polymer showed high viscosity at low concentrations, therefore it can easily form semisolid gels at low concentrations.[14]

d) **Transparency (Table):** All formulated gels were translucent except formulations F4 and F7 which were transparent. This can be attributed to both formulations F4 and F7 had the lowest concentration of polymer at 1% *w/w*, were shown as semifluid.

Grittiness (Table 2): All formulated gels showed no grittiness under a light microscope (40x magnification). Therefore, all formulated gels fulfilled the requirements of the absence of grittiness and particulate matter as anticipated for any topical formulation.[15]

pH measurements (Table 2): The pH values of all formulated gels ranged from 5.02 ± 0.02 to 6.38 ± 0.01. Since topical preparations will be directly applied onto the skin, their pH should be compatible with the skin pH. The skin should be weakly acidic ranged from pH 4.0 to 7.0 depending on location.[16] The results showed that the pH of all formulated gels was found to be within the normal pH range of the skin, which was considered acceptable to avoid the risk of skin irritation at the application site. Therefore, the results indicated that the acceptability of these formulated gels for topical use.

Viscosity studies (Table 2): The viscosity of various formulated acyclovir gels was found in the range of 200 to 45300 cPs. There was a nearly twofold increase in the viscosity from 1% *w/w* to 5% *w/w* of carbopol-940 concentration. The increase of HPMC concentration from 1% *w/w* to 5% *w/w* increased viscosity almost 227 times while the viscosity increased nearly 88 times from 1% *w/w* to 5% *w/w* of CMC Na concentration. Therefore, it was clear that the viscosity increased as the concentration of polymer in the gel formulations increased (p-value < 0.05).

Spreadability (Table 2): The larger is the spreading diameter, the more spreadable is the sample, and vice versa. The spreadability of all formulated gels ranged from 2.83 ± 0.153 cm to 7.83 ± 0.153 cm. However, there are no established guidelines on the ideal spreadability value. Therefore, it could be said that all the gels were easily spreadable. The spreadability of the gel formulations increased with decreasing polymer concentration. Statistical analysis using one-way ANOVA showed that the spreadability of the gel formulations was significantly affected by the polymer concentration (p-value < 0.05). Furthermore, it was particularly noteworthy that the spreadability of a gel had been shown to be related to its rheological characteristics. In brief, the viscosity of the gel formulations was increased as the polymer concentration increased. Meanwhile, the spreadability of the gel formulations was decreased.

Drug content uniformity (Table 2): All formulated gels were observed to contain 90.7 ± 0.460% to 99.1 ± 0.239% of acyclovir, which were lying within the pharmacopeial limits (90.0% to

110.0%) as stated in the USP.[17] The results showed that acyclovir was distributed evenly throughout the gel in all formulations. Therefore, the method employed to prepare gel formulations in this study was found suitable.[18]

In vitro drug release studies (Table 3)

Formulations F2, F5 and F8 were selected for further *in vitro* drug release studies due to their acceptable physicochemical characteristics to examine the effect of different polymer types on the release of acyclovir from the prepared gel formulations at the same polymer concentration (3% *w/w)*. The results showed that cumulative percentage drug release was highest for formulation F8, followed by formulation F5 and formulation F2 with the value of 96.21 ± 0.92%, 93.57 ± 0.77% and 72.61 ± 2.39% respectively at the end of 5 hours (Table 3 and Figure 1). One-way ANOVA analysis showed that the release of acyclovir from the prepared gel formulations was significantly affected by the type of polymer (p-value < 0.05). From this study, it was found out that the acyclovir gel formulated by using CMC Na showed maximum drug release rate over 5 hours.

Formulation F8 (3% *w/w* CMC Na) was selected as optimised formulation due to better cumulative drug release over 5 hours compared to formulations F2 and F5. Therefore, formulation F9 (5% *w/w* CMC Na) was further studied for *in vitro* drug release to investigate the effect of the concentration of polymer on the release of acyclovir from the prepared gel formulations. The results showed that the cumulative percentage drug release was 96.21 ± 0.92% for formulation F8 and 57.00 ±

3.07% for formulation F9 at the end of 5 hours (Table 1 and Figure 2). It was found that the release of acyclovir from the prepared gel formulations increased as the concentration of CMC Na decreased from 5% to 3%. One-way ANOVA analysis showed that the release of acyclovir from the prepared gel formulations was significantly affected by the concentration of polymer (p-value < 0.05).

It was clear from the above analysis that the drug release increased with decreasing polymer concentration. On the other hand, viscosity decreased as the concentration of polymer decreased. Therefore, the viscosity was inversely proportional to the release of acyclovir from the prepared gel formulations.[19] Formulation F9 showed lower drug release as compared to formulation F8 attributed to its higher concentration of polymer. The higher the polymer concentration in a gel formulation, the more rigid the three-dimensional network structure of the gel system, and the greater its viscosity, hence, the lower the drug release rate.[19] This may be due to the drug was entrapped in the smaller polymer molecules at the higher polymer concentration, causing a greater resistance to the diffusion of drug molecules through the gel matrix, thus decreased drug release rate.[19] Besides, the tortuosity of the gel matrix increased with increasing polymer concentration may be another possible cause for decreased drug release rate since the drug molecules have to travel a longer pathway to diffuse through the gel matrix.[14]

Analytical method validation (Table 4-5)

Determination of the wavelength of maximum absorbance

The UV scan of the working standard solution between 200 to 700 nm displayed the absorption maximum (λ max) at 250 nm for acyclovir. Therefore, the same λ max was used as the working wavelength for further measurements of the drug.

Robustness

The typical RSD for UV analysis is usually not more than 2%.[20] The results of the robustness test were within the acceptable range as shown in Table 4, indicated that the absorbance remained unaffected by small variation. Therefore, the proposed UV-Vis spectrophotometric method was considered as robust.

Linearity and range

Linearity was evaluated by using the least square regression method. The acceptance criteria for the coefficient of determination (R^2) should be ≥ 0.98.[20] As shown in Figure 3, The linear regression equation was obtained as $y = 0.0614x - 0.0139$ and the R^2 for the calibration curve was found to be 0.9996. Therefore, the high R^2 value ($R^2 \geq 0.98$) indicated clear correlations between the acyclovir concentrations and their absorbance within the test ranges.

Specificity

The UV spectra of the placebo showed no peak at the specific wavelength of acyclovir. However, the absorption peak of the selected acyclovir gel formulations at 250 nm was unchanged in the presence of the other excipients in the sample, indicated no effects of the excipients on the UV absorption of acyclovir. Therefore, it can be said that the proposed UV-Vis

spectrophotometric method was specific for the determination of acyclovir in topical gel formulations.

Accuracy

The acceptance criteria for the percent recovery should be 98% to 102% and the RSD is ≤ 2%.[12] The results showed that the mean percent recoveries for lower (8 µg/mL), intermediate (10 µg/mL) and higher (12 µg/mL) concentrations were found to be 99.27%, 99.33% and 99.43% respectively as shown in Table 5. Therefore, the proposed UV-Vis spectrophotometric method showed good accuracy because the results were within the limits with their low RSD values (RSD < 1%).

Precision (Table 6-7)

The acceptance criteria for both inter-day and intra-day precision tests should have a statistical RSD ≤ 2%.[12] The results showed that the RSD values of the four selected formulations were observed to be within the acceptable range for both of these tests as shown in Table 6 and Table 7. Therefore, the proposed UV-Vis spectrophotometric method was precise, reproducible and repeatable.

Conclusion

In the present study, acyclovir gels were successfully formulated by using three types of polymers as gelling agents with different concentrations, namely CMC Na, HPMC and carbopol-940. Viscosity studies showed that the viscosity of the gel formulations increased as the polymer concentration increased. Spreadability studies showed that the spreadability of

the gel formulations was significantly affected by the polymer concentration (p-value < 0.05). Moreover, the spreadability of the gel formulations decreased with increasing the polymer concentration. In short, the viscosity of the gel formulations increased as the polymer concentration increased. Meanwhile, the spreadability of the gel formulations was decreased. Furthermore, the *in vitro* drug release studies showed that the release of acyclovir from the prepared gel formulations was significantly affected by the type and concentration of polymer (p-value < 0.05). Among all prepared gel formulations, formulation F8 containing 3% *w/w* of CMC Na was selected as optimal gelling agent in acyclovir gel formulation due to its desired physicochemical properties and it showed the highest acyclovir *in vitro* release rate of 96.21 ± 0.92% over 5 hours. Therefore, it can be concluded that the topical acyclovir gels formulated in this study could be an alternative option for the effective management of HSV skin infections.

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