Original Article

FORMULATION AND EVALUATION OF TOPICAL PENTOXIFYLLINE-HYDROXYPROPYL METHYLCELLULOSE GELS FOR WOUND HEALING APPLICATION

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ABSTRACT

Objectives: Oral pentoxifylline shows modest, non-significant effect on the healing rates of chronic wounds. The present research aimed to formulate topical pentoxifylline-hydroxypropyl methylcellulose (HPMC) gels and evaluate their physico-chemical properties, *in vitro* release characteristics and *in vivo* wound healing effects.

Methods: Six gel formulations containing pentoxifylline (F1-F6) were prepared using HPMC with varying grades and concentrations. The physicochemical properties of gel formulations were evaluated in term of drug content, spreadability, rheological properties, swelling, and release characteristics. The efficacy of optimized formulation was further evaluated using *in vivo* excision wound models in rats.

Results: The spreadability, flow index and swelling percentage of gel formulations ranged 10.71-12.24 gcm/sec, 0.33-0.91 and 148.61-8011.61%, respectively. The rheological study of the prepared formulations exhibited pseudoplastic behavior, which is a characteristic feature of topical gels. Swelling results of F5 and F6 deduced that the cross-linked structures were formed between the polymeric chains. The *in vitro* drug release profiles of all formulations were found to be followed Higuchi model. The *in vivo* evaluation performed using rat excision wound model showed significant difference (P < 0.05) in the percentage reduction of wound size between treatment and control groups. The treatment group exhibited complete healing by day 13 as compared with day 15 in the control group.

Conclusions: These findings indicated that F5 gel formulation had demonstrated effective release profile for pentoxifylline. The *in vivo* results confirmed that F5 has pronounced wound healing effects when employed topically.

Keywords: Hydroxypropyl methylcellulose, Pentoxifylline, Topical gel, *In vitro* drug release, Wound healing.

INTRODUCTION

Wounds or loss of the integrity of large areas of the skin as a result of injury or illness may lead to major disability or even death. Pentoxifylline, a synthetic methylxanthine, has been used to aid the management of intermittent claudication and wounds [1-4]. Pentoxifylline was introduced in 1984 as a medication for the prevention and symptomatic treatment of patients with intermittent claudication in chronic occlusive arterial disease [1,2]. Pentoxifylline was found to accelerate the wound healing process in animal models and patients with diabetic ulcers and venous ulcers [3, 4]. However, it was reported that the oral route of administration of pentoxifylline had a non-significant effect on the healing rates of chronic wounds because of its low bioavailability since it is subjected to extensive hepatic first-pass metabolism [5,6]. Hence, suitable topical delivery system could be developed to administer pentoxifylline directly at the site of wound to overcome the drug's limitations via oral route administration.

Topical drug delivery is an useful route of administration for the drug because it offers easy way for patients to use, serves as painless route for drug application and prevents the hepatic first pass metabolism of the drug [7,8]. Within the major groups of topical drug delivery system, the use of topical gel formulation has expanded in pharmaceutical field due to its favorable properties such as greaseless, easily spreadable, easily removed, thixotropic, emollient and water soluble properties to users. [2, 9-16]. Hydroxypropyl methylcellulose (HPMC) is cellulose ethers which has been used as the hydrophilic gel matrices for controlled release oral delivery due to its ability to release an incorporated drug by control of swelling and crosslinking [14,17]. Furthermore, HPMC is recommended in the drug formulation and delivery because of its non-toxic nature, ease of compression, good bioadhesive properties

and accommodation to high levels of drug loading [18-20]. A recent study reported that HPMC in topical formulations of recombinant human vascular endothelial growth factor (rhVEGF) enhanced release of rhVEGF and therapeutic activity. [21]. In light of current knowledge, several researchers have been focusing on developing formulations by varying concentrations of polymer and studying their *in vitro* release profile [22, 23]. However, there is lacking knowledge in the study of active substance or drug released from the formulations developed using the different viscosity grades of polymer. Based on the literature search, there is only a study conducted by Mitchell and Balwinski which has predicted that drug release variability over the United States Pharmacopeia (USP) viscosity ranges [24].

Herein, the aim of this present study was to develop a topical delivery system that comprises pentoxifylline with a hydrogel base prepared from various viscosity grades and concentrations of HPMC, evaluate the release mechanisms and demonstrate the beneficial effects in treating excisional skin wounds in rat model.

MATERIALS AND METHODS

Chemical

Four different grades of HPMC were purchased from Sigma-Aldrich Sdn. Bhd. The specification was as listed in Table 1.

Preparation of pentoxifylline-HPMC topical gels

The required amount of ultrapure water was heated to 90°C. Then, HPMC powder was slowly dispersed in the pre-heated water. The entire HPMC solution was stirred until a homogenous appearance was attained. The solution was then left overnight in the refrigerator until a transparent gel was formed.

Table 1: Specifications of hydroxypropyl methylcellulose (HPMC) powders used

Sample	Molecular weight, M_n (kDa)	Viscosity, 2 wt% aqueous solution at 20°C (cP)
H1	10	6
H2	86	2600-5600
H3	90	15000
H4	120	100000

Pentoxifylline were purchased from Sigma-Aldrich Sdn. Bhd. All other chemicals were purchased from Merck Sdn. Bhd. These chemicals were used as received.

The topical gel formulations of pentoxifylline-HPMC were prepared by mixing pentoxifylline with HPMC gel under continuous stirring at ambient temperature until all pentoxifylline powder had been homogenously dissolved. The detailed compositions of pentoxifylline-HPMC formulations were given in Table 2.

Table 2: Formulations of pentoxifylline-HPMC gel

Formulation of pentoxifylline-HPMC	Percentage weight per weight (wt%)					
	H1	H2	H3	H4	Pentoxifylline	Water
F1	1	-	-	-	1	98
F2	-	1	-	-	1	98
F3	-	-	1	-	1	98
F4	-	-	-	1	1	98
F5	-	-	-	2	1	97
F6	-	-	-	3	1	96

Homogeneity test

The homogeneity of each gel was tested by visual inspection after the gels were set in the container. They were examined for their physical appearance and presence of any aggregates.

Drug content determination

A series of formulations were prepared and the absorbance was recorded using Ultraviolet-visible spectrophotometer (Perkin-Elmer, Malaysia) at 274 nm wavelength using Phosphate buffered saline buffer (PBS) solution with pH 7.4 as blank. A standard curve of absorbance vs. concentration of drug in PBS was plotted and the drug content of each formulation was determined.

Spreadability test

The spreadability of gel was determined by wooden block and glass slide apparatus. The gel was sandwiched between two galss slides with one of the slide fixed on the block. The top plate was subjected to pull a weight of 80 g. It was noted for upper slide separate completely from the fixed slides. The spreadability was calculated using the following equation:

$$S = M \times \frac{L}{T} \rightarrow \textbf{Eq. 1}$$

In which S is the spreadability of gel, M is the weight in the pan which tied to the upper slide, L is the length moved by the glass slide and T is the time taken to separate the slide completely from each other.

Rheological study

The rheological properties of gels were evaluated at ambient temperature using a Rheolab MC1 brand rheometer. The mode of experiment was viscometry sweep, employing cylinder-cone method, Z2 DIN measuring system and torque element 55,000 μNm. The rheological data obtained were fitted to the power law model as shown in equation 2.

$$\tau = K\dot{\gamma}^n \rightarrow \textbf{Eq. 2}$$

In which τ is the shear stress, $\dot{\gamma}$ is the shear rate, K is the consistency index (Pa. s.^{n}.) and n is the flow behavior index.

Swelling index

About 1 g of gel was dissolved in 25 ml water kept for 24 hours. The solution was filtered using Millipore filter (0.45 μm*). The swollen gel was reweighed and the data collected was used to calculate the swelling index using the following equation 3.

$$\text{Swelling Index} = \frac{W_1 - W_2}{W_2} \times 100 \rightarrow \textbf{Eq. 3}$$

In which W_1 was the weight of swollen gel after 24 hours and W_2 was the original weight of gel at zero time.

In vitro drug release study

The drug release of pentoxifylline from all the formulations across artificial cellulose membrane was investigated by using Franz diffusion cells (PermeGear, USA). About 1 g of gel formulation was placed on a 0.45 μm pore size, cellulose acetate membrane in the donor compartment. The receptor compartments were filled with 25 mL of PBS pH 7.4 which was maintained at 32 ± 0.5°C. Aliquots of the receptor medium were withdrawn and replaced with the same amount of fresh receptor medium at time intervals of 1, 2, 4, 6, 8, 12 and 24 hours. The withdrawn aliquots were analysed by ultraviolet–visible spectrophotometer (Perkin-Elmer, Malaysia) at 274 nm wavelength. The cumulative percentage of drug release was calculated and plotted against time.

In vitro drug release kinetics

The release characteristics of gel formulations were determined by fitting the release data to the following equations of zero-order, first order and Higuchi models which are given by equations 4, 5 and 6, respectively.

$$Q = k_0 t \rightarrow \textbf{Eq. 4}$$

$$\ln(Q_0 - Q) = \ln Q_0 - k_1 t \rightarrow \textbf{Eq. 5}$$

$$Q = k_2 \sqrt{t} \rightarrow \textbf{Eq. 6}$$

In which Q is the amount of drug released at time t and Q_0 is the initial amount of drug; k_0, k_1, k_2 are the rate constants of zero order, first order and Higuchi models respectively.

In vivo wound healing study

From the optimization study, the F5 formulation was selected as it has the best spreadability and good release characteristics. Male Sprague-Dawley rats weighing 200 – 250 g were used in this study. Upon arrival, all the rats used were without defects in general appearance. The rats were kept in the animal holding facility in the International Medical University (IMU) throughout the experiment. Each rat was housed separately in the individually ventilated cages, with food and water given *ad libitum*. All the experimental protocols were approved by the IMU Joint-Committee of Research and Ethics Committee, International Medical University, with the IRB ref no. BMSc 4.4/JCM-49/2011. The *in vivo* study was evaluated using twelve rats with excision wound model. On the day of wound creation (Day 0), all the rats were anesthetised by intraperitoneal injection of ketamine at 100 mg/kg and xylazine at 10 mg/kg. After shaving the backs of the animals, a circular full-thickness excision wound of 1 cm × 1 cm was created at the interscapular area, by removing the skin layer extending from the epidermis until the

subcutaneous tissue layer. All the wounds were left uncovered and no additional medications were administered throughout the experiment. Twelve rats were randomly divided into two groups.

Control group: Untreated with no gel formulation were given.

Treatment group: Treated with medicated gel topically once daily.

Wounds were photographed using a digital camera (Canon PowerShot SD790 IS, Japan) on day 0 and day 10. All photographs were taken from a fixed distance of the wound (see Fig. 3). The excision wounds were evaluated for the percentage of reduction of wound size, starting from Day 0, Day 5 and Day 10, using equation 7. The duration of complete wound closure was also recorded for each experimental group. The data collected was subjected to statistical analysis for comparing the differences in wound healing outcomes between the treated groups and the untreated group.

The analysis was performed using SPSS 17 version 12.0 for statistical t-tests. Analysis outcomes showing P <0.05 were considered statistically significant. This statistical significance would indicate the treatment has a definite effect on the healing outcomes.

Percentage reduction of wound size (%)

$$= \frac{\text{Wound area on Day 0} - \text{Wound area on Day n}}{\text{Wound area on Day 0}} \times 100\% \rightarrow \textbf{Eq. 7}$$

RESULTS AND DISCUSSIONS

All the gel formulations showed clear, transparent and good homogeneity with absence of lumps. As shown in the Table 3, the drug contents of all formulations were complied with the acceptable range of 99% - 100%. The spreadability of F5 was the highest, which is 12.04 g. c.m./sec. This indicated that F5 showed the best spreadability among these formulations.

Table 3: Drug contents, spreadability and rheological properties of HPMC-pentoxifylline gel formulations

Formulation	Drug Content (%)	Spreadability (g. c.m./sec)	Consistency index (k)	Flow index (n)	Maximum viscosity
F1	99.99	11.32	0.16	0.33	0.30
F2	99.88	10.91	0.25	0.91	0.27
F3	99.91	10.91	0.50	0.88	0.56
F4	99.71	10.71	4.81	0.58	7.21
F5	99.94	12.04	50.59	0.49	83.20
F6	99.95	11.44	194.14	0.55	302.00

In the rheological study, all gels showed non-Newtonian fluid behavior. From Table 3, it was found that the flow indexes of all formulations were less than 1, signifying the presence of shear-thinning or pseudoplastic behaviors. This behavior can be interpreted as indicating the presence of weak attractive forces between the polymer chain molecules, where these polymer chain molecules formed entangle loops with each other, forming temporary junctions at entanglement points. When the high shearing force was applied in the study, the polymer fluid would initially show a solid-like resistance against point network. Then, the entangled segments would elastically stretch and eventually the molecules started to disentangle and flowed in the direction of the shearing force. As shown in Table 3, it was found that the values of consistency index, k, and maximum viscosity increased with an increase of molecular weight and concentration of HPMC as seen in the formulation of F1 to F6. This explained the presence of interloops and entanglements in the HPMC solution increased with an increase in the molecular weight and concentration of HPMC in the formulations.
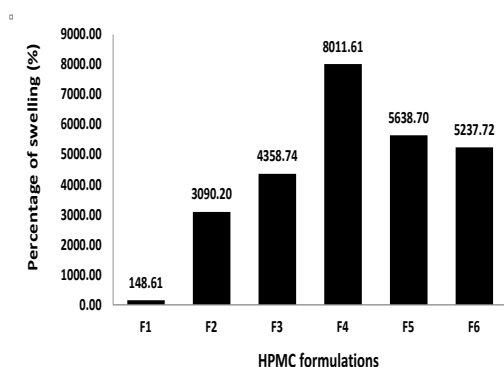
weight from F1 to F4. This indicated that water absorption and retention abilities of gels gradually increase from F1 to F4. The good water absorption property of HPMC H4 gel (as in F4) could be used as a suitable wound dressing material for topical applications. Additionally, Gwon*et al.* have demonstrated a correlation between the water absorption ability and the exudate absorption capacity of hydrogel wound dressings [25]. Hence, it can be deduced that HPMC H4 gel (F4) might have better capacity to absorb excess wound exudates. From Fig. 1, the swelling percentage decreased from F4 to F6 as the concentration of HPMC H4 gel increased from 1 wt% to 3 wt%. This implied an increase of crosslink structures was formed in the HPMC gel network as the concentration of HPMC increased. These crosslink polymer network restrict the extensibility of the polymeric chains which induced by the swelling of fluids. In fact, these crosslinked three-dimensional structures are favorable in regulating the diffusion of drug molecules across the gel network thus allows for their possible use as drug carriers for controlled release applications [2].

***In vitro* drug release study**

The *in vitro* release profile of topical gel was represented in Fig 2. It was found that all tested formulations were able to control the release of pentoxifylline for up to 24 hours. The release of pentoxifylline from its different formulae can be ranked in the following descending order: F1> F2> F3> F5> F4 > F6; where the amounts of drug released in24hours were 86.09%, 78.51%, 77.28%, 71.56, 71.47% and 70.48% respectively. A.l.l the formulations revealed more than 70% of pentoxifylline was released in 24 hours. The slowest release rate was demonstrated by formulation F6 which released only 70.48%. This result can be explained by more entangled loops formed when the polymer concentration increased in F6. These entangled loops helped in entrapping large amount of drug and simultaneously the release of drug become slower due to the mobility of drug solute become more restricted within these entangled loops.

Table 4 presented rate constant and correlation coefficient of different kinetic models for pentoxifylline-HPMC gel systems. The plots of percent release against time for all the formulations were not linear which indicating that pentoxifylline release did not follow the zero order. However, first order and Higuchi order plots showed linear release pattern. The release patterns were further confirmed by checking the correlation coefficient of the plots. The calculated correlation coefficients of the Higuchi plots were ≥0.95, whereas the

Fig. 1: Swelling percentage of HPMC with different molecular weights and concentrations

The swelling percentage of HPMC after being immersed in water for 20 hours is graphically presented in Fig. 1. The results showed that 1 wt% HPMC gels swelled rapidly with the increase of its molecular

first order plots were <0.95. This implied that the best-fit release of pentoxifylline from the prepared gel formulations followed Higuchi order.
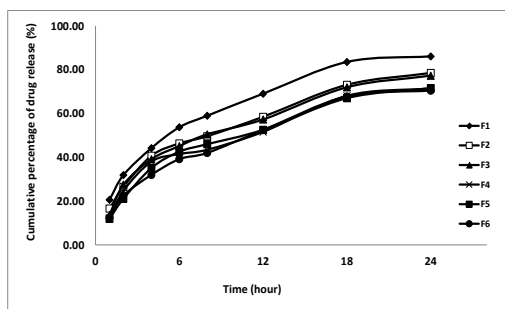

Fig. 2: Cumulative percentage of pentoxifyllinerelease from gel formulations

In vivo wound healing study

In this study, it was found that the progress of healing started from the first day as the diameter of wound size started to reduce. As shown in both Fig. 3 and Table 5, at the end of the treatment period (10 days), the percentage reduction of wound size for the pentoxifylline gel formulation-treated lesions were higher than control group, with a difference of about 8%.

Statistical t-test analysis of the data (percentage reduction of wound size and number of days required for complete healing) carried out between the treatment group and the control group indicated that there was a significant difference (P values < 0.05) between the wound diameter on day 5 and 10 between both groups (see Table 5). Significant difference was also detected for the total number of days required for complete healing in both of the control and treated groups. This result suggested that pentoxifylline-HPMC gel formulation demonstrated beneficial therapeutic effect in wound healing.

Table 4: Correlation coefficient of different kinetic models for pentoxifylline-HPMC gels

Formulation	Zero-order kinetics		First-order kinetics		Higuchi model	
	K_0	R^2	K_1	R^2	K_H	R^2
F1	4.68	0.11	0.09	0.91	19.57	0.95
F2	4.13	0.27	0.07	0.88	17.16	0.97
F3	4.07	0.30	0.07	0.88	16.89	0.97
F4	3.75	0.35	0.06	0.84	15.54	0.96
F5	3.75	0.43	0.06	0.87	15.51	0.97
F6	3.69	0.51	0.06	0.89	15.17	0.99

Table 5: Percentage reduction of wound size and day for complete healing

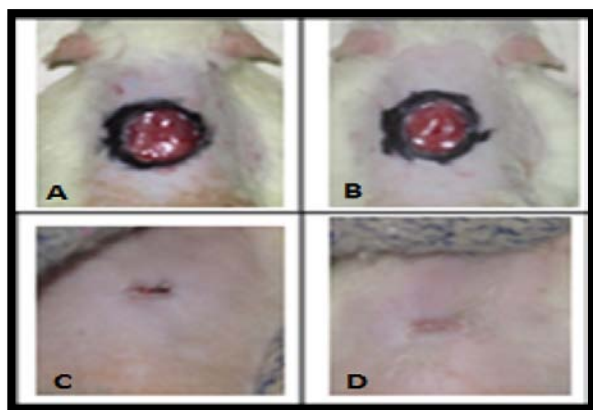| Animal	Control group			Treatment group		
	Percentage reduction of wound size (%)		Day for complete healing	Percentage reduction of wound size (%)		Day for complete healing
	Day 5	Day 10		Day 5	Day 10	
Average	34.65%	87.98	15.00	51.88	95.97	13.00
P-value	-	-	-	<0.05	<0.05	<0.05

Fig. 3: Photographic shots of wounds in control group; A (day 0), C(day 10) and treatment group; B(day 0) and D (day 10)

CONCLUSION

In this work, attempts were made to prepare different topical pentoxifylline-HPMCgel formulations with HPMC varying molecular weights and concentrations. All gels have good homogeneity, spreadability and followed Higuchian diffusion fashion. Swelling of F5 and F6found to be closely related to the availability of more crosslinked three-dimensional structures. However, F5 possessed better spreadability and higher cumulative release amount at 24 hours than F6. Hence, it is well justified to conclude that F5 was identified to be the most suitable formulation for topical application

in our current study. Lastly, *in vivo* study proved that F5 promoted wound healing when compared with control group.

CONFLICT OF INTEREST STATEMENT

Authors declare that they have no conflict of interest in the research.

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