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Film forming gel for treatment of oral mucositis: *In vitro* studies

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Abstract

Oral mucositis is one of the main complications of non-surgical cancer treatments. The present work focuses on the treatment or reduction of oral mucositis by using combined mechanism by formation of physical barrier by forming a film to cover the oral ulcer and use of therapeutic agents, such as diclofenac sodium and ofloxacin separately or in combination. The selected polymers for film forming gel formulations are Hydroxypropylcellulose (HPC), hydroxypropyl methylcellulose (HPMC), sodium carboxymethylcellulose (Na CMC) and carbopol 940 (CP). The residence time in simulated buccal saliva was between 5.5 to 6 hours for all formulations. The in-vitro release data of the investigated drugs from the prepared formulations followed zero-order and diffusion mechanism. The permeability studies data revealed that diclofenac sodium showed higher permeability from Na CMC/CP (2:0.3%) than from HPMC 4%, while in case of ofloxacin higher permeability was shown from Na CMC/CP (2:0.3%) than from HPMC/HPC (2:3%). The permeation parameters for diclofenac sodium and ofloxacin in their combination do not depend on either viscosity or pH, they depend on the type of polymers used.

Keywords: Mucositis; Film-forming gel; Rheology; *In vitro* studies.

Introduction

Mucositis induced by anti-neoplastic drugs is an important, dose-limiting, and costly side effect of cancer therapy. The ulcerative lesions produced by mucotoxic chemotherapy are painful, restrict oral intake and, importantly, act as sites of secondary infection of oral flora [1].

Previously published data [2-8] have pointed out that the prophylaxis and treatment of oral mucositis during cancer therapies remain an unsolved problem. Pretreatment assessment of oral cavity hygiene and mouthwashes seem to be effective in preventing the onset of oral mucositis [9]. Some therapeutic agents, such as benzydamine, imidazole antibiotic, triazolic antimycotic and povidone iodine have shown some clinical evidence of their efficacy in reducing oral mucositis.Bioadhesive polymers appear to be particulary attractive for the development of drug delivery systems to improve intraoral administration [10-14] and reduce the frequency of application and the amount of drug administered. Gels and films may be most suitable for this type of application and they are able to cover a wide area of mucosa for both drug delivery and physical protection [15, 16]. The aim of this study was to develop a film forming gel system containing diclofenac sodium, ofloxacin and their combination for prophylaxis and/or treatment of oral mucositis. Film forming gel formulations were prepared using mucoadhesive polymer to produce a

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physical barrier around the ulcers and form a medicated film for delivery of either diclofenac sodium or ofloxacin to treat the formed ulcer.

Materials and methods

Materials

Diclofenac sodium (Delta pharmaceuticals, Cairo, Egypt), ofloxacin (El Kahira Co. Cairo, Egypt), hydroxypropylcellulose MF (Kolmar, California, USA), hydroxyl propylmethylcellulose E4M (Dow Chemical Co., USA), sodium carboxymethylcellulose (BDH Chemicals, Ltd, Poole, UK), carbopol 940 (Sedico pharmaceuticals, Cairo, Egypt). All other chemicals were of analytical reagent grade.

Formulation and preparation of the gels

The gels were obtained using different mucoadhesive polymers, diclofenac sodium, ofloxacin (active compounds) and their combination were incorporated into the formulations. Composition of the different gels is shown in table 1.

Table 1. Composition (% w/w), pH, residence time and bioadhesive force of the film forming gel.

Formula Code	Polymer Type	Polymer Concentration	pH	Residance Time (hr)	Bioadhesive Force (cm)
1	HMPC	4%	6.76	6	1.5
2	HMPC/HPC	2:3%	6.42	5.5	4.5
3	HMPC/HPC	3:3%	6.32	5.5	3
4	HMPC/HPC	4:3%	6.71	6	2
5	HPC	16%	7.1	5.5	5
6	HPC/CP	4:0.3%	7.7	6	2.5
7	Na CMC/CP	2:0.3%	7.3	6	1.5

HPMC, Hydroxyprpyl methyl cellulose; HPC, Hydroxyprpyl cellulose; CP, Carbopol 940; Na CMC, Sodium carboxymethyl cellulose.

The polymers were dispersed in 60 % ethanol. Incorporation of 0.1%w/w diclofenac sodium, or 0.5% w/w ofloxacin or their combination was performed by dispersing them in water at 50-60 ˚C and then pouring this dispersion onto the polymer mixture under slow stirring for 20 min. Propylene glycol was incorporated to increase the solubility of ofloxacin. Dispersion was mixed using a magnetic stirring bar till a clear transparent gel free from air bubbles was formed. pH measurement was carried out using pH meter 3510, Jenway, Essex, (England).

Residence time of the gels

The time taken by the film formed from different gel formulations, after evaporation of the vehicle; to dissolve was monitored for 6 hours in simulated saliva by applying 1 gm of the gel on the centre of a watch glass, making a circle of 3 cm in diameter left until dryness. Six ml of simulated saliva were added on two portions to simulate the buccal cavity.

Bioadhesivness of the gels

The force required to overcome the attraction force between the surface and the sample (adhesiveness) was measured by the plate agar method which showed the bio-adhesiveness of the gel [17]. A plate containing agar (2% w/v) of 5 cm diameter was prepared, 0.5 gm of the gel was placed on the centre of the agar plate, making a circle of 5 mm in diameter. The plate was slanted at 30˚ for 1 hour and the longest distance moved by the gel was measured at room temperature.

Rheological properties of the gels

The rheological properties of film forming gel were evaluated using Bohlin Gemni 200 Rheometer at 25˚C using 1 g of a sample. In order to identify the flow behavior of each gel base, viscosity was determined at different shear rates to generate a complete flow curve by plotting viscosity versus shear rate.

Fourier transform infrared spectrophotometry (FTIR)

FTIR spectra of the polymer used, diclofenac sodium, ofloxacin alone and in the gel formulations were recorded on Nicolet Avatar 380 spectrometer. Attenuated total reflection method was adopted using a diamond crystal as an internal reflection element.

Gel stability

Stability of the gel formulations was also investigated. The organoleptic properties (color, odor), pH, and drug content of the gels stored at 40 ˚C were examined for 3 months. Drug content for diclofenac sodium and ofloxacin alone was measured spectrophotometrically at 277 and 290 nm respectively. Their combination was measured using HPLC method at 280 nm.

***In vitro* drug release**

The in vitro release data were fitted to different models; the following mathematical equations were used:

Zero-order kinetic model

$$M_0 - Mt = k_0 t \quad (1)$$

First-order model

$$\text{Ln } M_0 / Mt = K_1 t \quad (2)$$

Higuchi model

$$Mt = K_H t^{1/2} \quad (3)$$

where M_0 and Mt correspond to the amount of drug taken at time zero, or dissolved at a particular time t. k_0, k_1 and K_H the release kinetic constants obtained from the linear curves of the zero-order, first-order and Higuchi model, respectively [18,19] .

The release of drugs from gels was performed through a dialysis membrane (Fischer cellophane membrane 30/32), which was placed in continuously stirred 50-ml simulated saliva (pH 7.5) [disodium hydrogen phosphate (2.38g/L), potassium dihydrogen phosphate (0.19g/L) and sodium chloride (8g/L)] at 37±0.5 ˚C [20]. At appropriate time intervals, a 5-ml sample was withdrawn and replaced with the same amount of fresh simulated saliva.

***In vitro* permeation study**

Franz diffusion cell with a 3.14-cm^2 diffusion area and 16-ml receptor volume were used to study the permeability of the incorporated drugs. The excised rat peritoneal membranes were placed between the donor and receptor compartments of the cells. Approximately16 ml of simulated saliva (pH 7.4) was placed in the receptor compartment. Its temperature was maintained at 37 ± 0.5˚C and it was stirred at 50 rpm throughout the experiment. The donor compartment contained 1 g of the sample. At appropriate time intervals, a 2-ml sample was withdrawn and replaced with the same amount of simulated saliva. The diclofenac sodium concentration in the samples was determined spectrophotometrically, using UV spectrophotometer 6315, Jenway, Essex, (England) at 277 nm against a blank prepared with the permeated formulation without the drug [21]. Ofloxacin concentrations was determined spectrophotometrically at 290 nm against a blank prepared with the permeated formulation without the drug (Park et al., 2000), while the combined samples was determined using a modified HPLC method at 280 nm [22]. Three replicates of each experiment were performed.

Results and discussion

Residence time of the gels

Residence time in simulated buccal saliva shown in Table 1 is 6 hours for formulations of Na CMC/CP (2:0.3%) and HPMC (4%) while HPC (3%) in combination with HPMC (2%) and (3%), and HPC/CP (4:0.3%) is 5.5 hours. Finally, HPMC/HPC (4:3%) and HPC (16%) is only 5 hours.

Bioadhesivness of the gels

The bio-adhesiveness data shown in table 1 revealed that the most adhesive gels are Na CMC/CP (2:0.3%), HPMC (4%), and HPC/CP (4:0.3%) followed by HPC (3%) in combination with HPMC (3%) and (4%). The least adhesive gels are HPMC/HPC (2:3%) and HPC (16%).

Rheological properties of the gels

The rheological data is shown in figure1-3 revealed that all film forming gel formulations exhibited shear thickening behavior at low shear rates followed by shear thinning at high shear rates, except for HPC (16%) and HPMC/HPC (2:3%) showed Newtonian flow. The highest viscosity was recorded for formulations of HPMC (4%), HPMC/HPC (3:3%) and (4:3%), and Na CMC/CP (2:0.3%). Figure 4 -6 represent a relation between shear rate and shear

stress of different film forming gel. The formulations showed pseudoplastic flow.

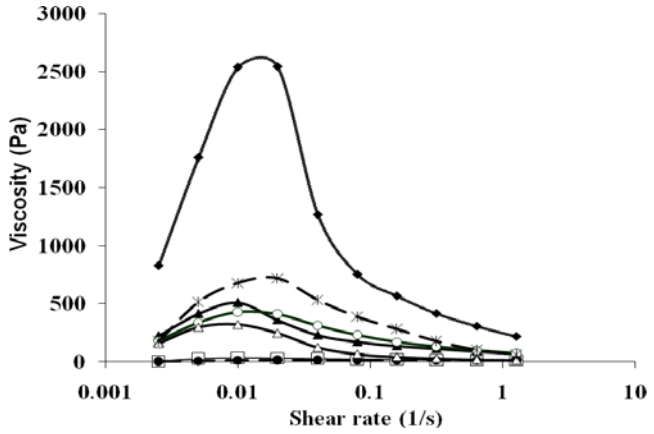

Figure 1. Viscosity of different film forming gels containing diclofenac sodium in HPMC 4% (♦), HPMC 2%: HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and NaCMC 2%:CP 0.3% (×) as function of shear rate.

Fourier transform infrared spectrophotometry (FTIR)

The IR data revealed that there was no significant interaction between diclofenac sodium, ofloxacin and polymer used. The bands due to diclofenac sodium especially in the range 3600-3000 cm^{-1} are not clear in the spectrum of the diclofenac sodium and its combination with ofloxacin.

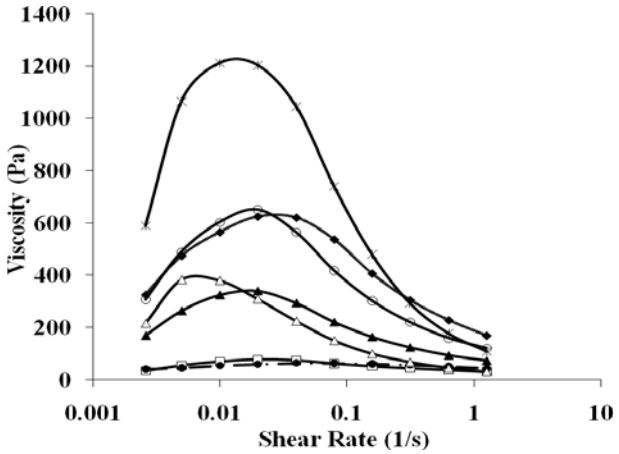

Figure 2. Viscosity of different film forming gels containing ofloxacin in HPMC 4% (♦), HPMC 2%: HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×) as function of shear rate.

This could be explained on the basis that diclofenac sodium band overlapped with the broad O-H band of hydroxypropylmethylcellulose appeared in this region. On the other hand, ofloxacin does not interact in any way with the polymer used.

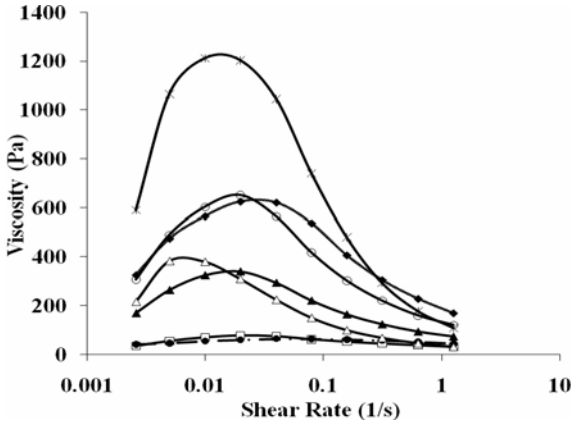

Figure 3. Viscosity of different film forming gels containing diclofenac sodium and ofloxacin in HPMC 4% (♦), HPMC 2%: HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×) as function of shear rate.

Gel stability

Stability studies were conducted at 40˚C. All the gel formulation tested remained stable after 3 months of storage at 40˚C.

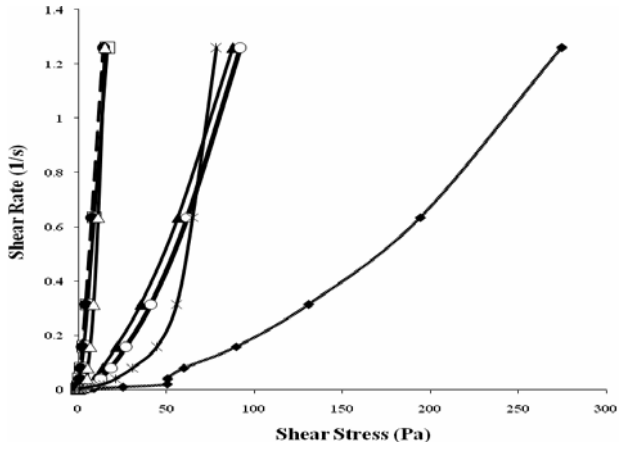

Figure 4. Rheogram of film forming gels containing diclofenac sodium in HPMC 4% (♦), HPMC 2%:HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×).

***In vitro* release study**

The kinetics of drug release was studied in different formulations. Preference of a certain mechanism was based on the correlation coefficient for the parameters involved. The kinetic analysis of the release data reveals that diclofenac sodium, ofloxacin and their combination in different film forming gel were released by zero order and diffusion mechanism for the 6 hours of the study. This could be attributed to; release was first from the gel applied, after that the release was from a thin film formed as the alcohol evaporated from the gel. The release of diclofenac sodium shown in figure 7 from film forming gel based on HPMC (4% w/w) and HPC (16% w/w) alone was higher than those containing both polymers (different concentrations of HPMC with fixed concentration of HPC (3% w/w)).

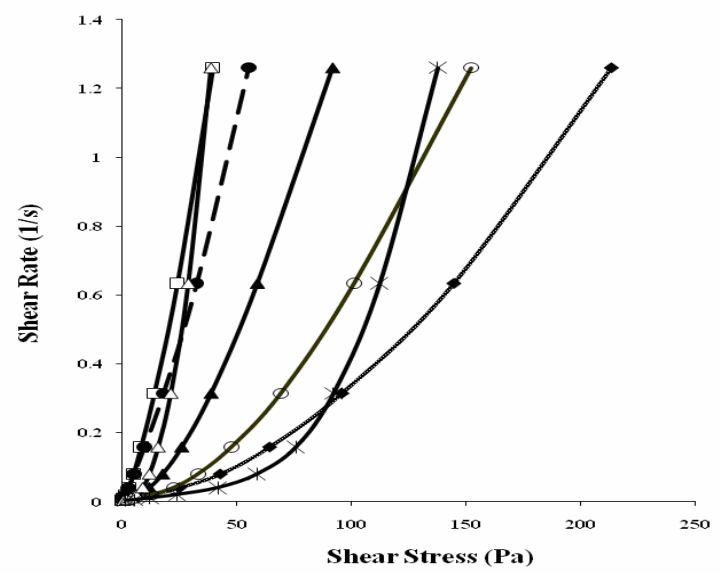

Figure 5. Rheogram of film forming gels containing ofloxacin in HPMC 4% (♦), HPMC 2%:HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×).

This may be due to the differences in wettability between HPMC and HPC which led to a decrease in the entrance of water through the polymer [23]. The release from HPC (16% w/w) alone (75.7%) was higher than its combination with carbopol (37.7%). This could be explained on the higher viscosity of HPC film forming gel in presence of carbopol (158.8 Pas) in comparison to HPC alone (16.72 Pas). The release from film forming gel composed of Na CMC/CP (2:0.3%) was the highest compared to other gel formulations (98.7%). This result is in agreement with the previous finding [23]. At pH 6.8, the Na CMC gel has a loose structure where the weak gel structure determines the ability of the polymer chains to disentangle from the polymer network and to dissolve. This results in faster erosion of the hydrogel and enhances drug release. The release of ofloxacin shown in figure 8 was high from HPMC/HPC (2:3%) due to the low viscosity of this formulation**.** On the other hand, the presence of carbopol in combination with HPC (4%) potentiated the rapid release of ofloxacin from the gel due to its high hydrophilicity, while HPC only retarded ofloxacin release. Also, the release from Na CMC/CP gel recorded a very high release percentage.

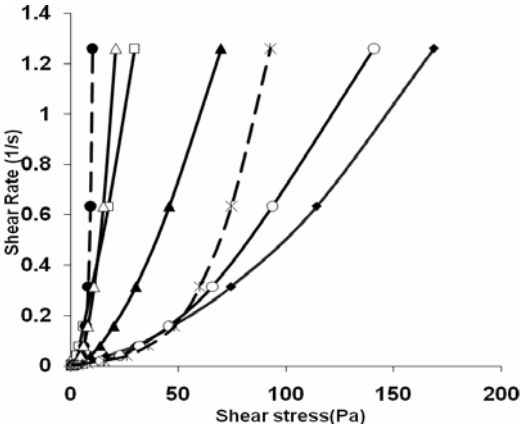

Figure 6. Rheogram of film forming gels containing diclofenac sodium and ofloxacin in HPMC 4% (♦), HPMC 2%:HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×).

The data presented in figure 9 revealed that the presence of ofloxacin decreased the cumulative percent released of diclofenac sodium from three formulations: HPMC 4%, HPC 16%, and Na CMC/CP (2:0.3%), while showing no effect on the rest of formulations. Also, it was observed that the presence of diclofenac sodium increased the cumulative percent released of ofloxacin shown in Fig.10 from four formulations: HPMC (4%), HPMC/HPC (3:3%), HPMC/HPC (4:3%) and HPC (16%), while decreasing the release from the rest of formulations.

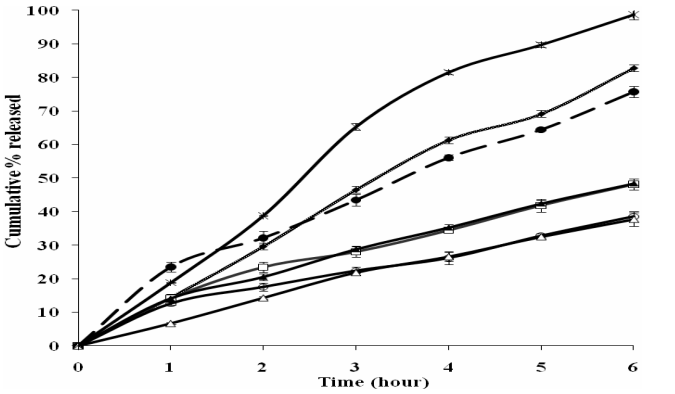

Figure 7: Release profile of diclofenac sodium (0.1%w/w) from different film forming gels in HPMC 4% (♦), HPMC 2%:HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×).

Permeability study

The formulations which gave good results with highest release percentage were selected for permeability studies. To calculate the permeation parameters of the Fick's law equation a graph of penetrated amounts vs. time was plotted. It is possible to calculate the steadystate flux (J) from the slope of the linear portion (2-6 hours) of the graph [24, 25].

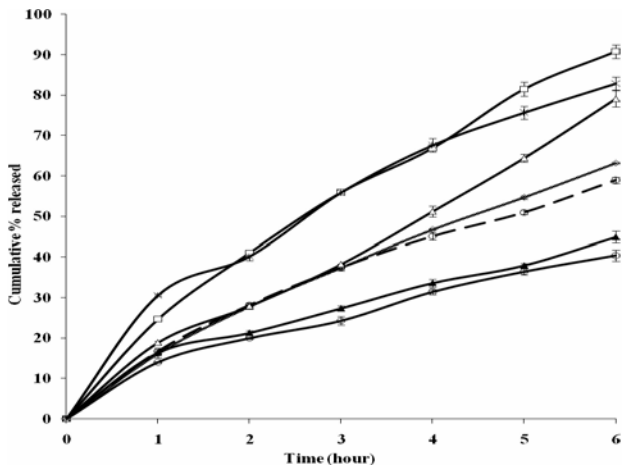

Figure 8: Release profile of ofloxacin (0.5%w/w) from different film forming gels in HPMC 4% (♦), HPMC 2%:HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×).

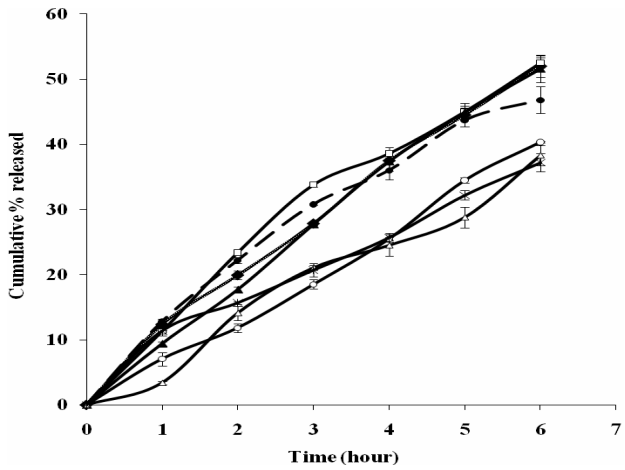

Figure 9: Release profile of diclofenac sodium (0.1%w/w) from its combination with ofloxacin (0.5%w/w) in different film forming gels in HPMC 4% (♦), HPMC 2%:HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×).

The permeability coefficient (Kp) was calculated from the steady-state flux and the applied concentration in the donor compartment (C donor) as follows:

Kp = J/C donor

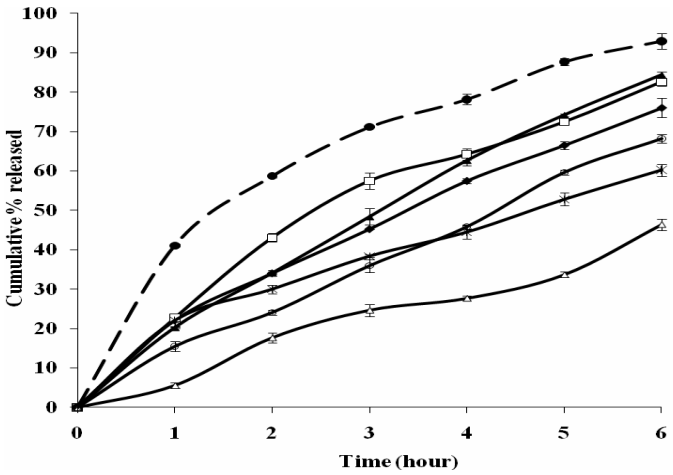

Figure 10: Release profile of ofloxacin (0.5%w/w) from its combination with diclofenac sodium (0.1%w/w) in different film forming gels in HPMC 4% (♦), HPMC 2%:HPC 3% (□), HPMC 3%:HPC 3% (▲), HPMC 4%:HPC 3% (○), HPC 16% (●), HPC 4%:CP0.3% (Δ) and Na CMC 2%:CP 0.3% (×).

Table 2. Permeation parameters of diclofenac sodium and ofloxacin delivered from different film forming gel formulations.

Formulation code	J (μg.cm^{-2}.hr^{-1}) ±SD				K_P(cm.hr^{-1}) x10^{-2} ±SD			
	Single		Combined		Single		Combined	
	Diclofenac Sodium	Ofloxacin	Diclofenac Sodium	Ofloxacin	Diclofenac Sodium	Ofloxacin	Diclofenac Sodium	Ofloxacin
HPMC 4%	8.6 (±0.003)	-	-	-	0.99 (±0.017)	-	-	-
HPMC2% HPC 3%	-	35.9 (±0.004)	22.1 (±0.02)	135.3 (±0.002)	-	0.76 (±0.015)	2.2 (±0.01)	2.65 (±0.015)
HPC 16%	-	-	1.14 (±0.017)	15.5 (±0.013)	-	-	0.12 (±0.0016)	0.37 (±0.11)
Na CMC 2% CP 0.3%	15.5 (±0.012)	340.2 (±0.015)	-	-	1.78 (±0.002)	6.53 (±0.02)	-	-

The flux and permeability coefficients of the formulations are given in Table 2. It is clear that there is a correlation between viscosity and permeation parameters such as HPMC (4% w/w) containing diclofenac sodium with viscosity value (828.2 Pas) has low permeability coefficient (0.99 x10^{-2} cm.hr $^{-1}$) in comparison with Na CMC/CP (2:0.3% w/w) formulation with permeability value (1.7 x10^{-2} cm.hr $^{-1}$) having viscosity equal to 177.1 Pas.

In case of HPMC/HPC (2:3% w/w) [pH=6.9] with low viscosity value (34.6 Pas) has low permeation parameter for ofloxacin (flux 35.9 μg.cm^{-2}hr^{-1}; Permeability coefficient (0.76 x10^{-2} cm.hr^{-1}) than the formulation of Na CMC/CP (2:0.3% w/w) [pH=7.8] with high viscosity (589.1 Pas). This could be attributed to the role of pH on the solubility of ofloxacin. In case of combined samples, there was a faster permeation of ofloxacin than diclofenac sodium which is attributed to the differences in solubility of both drugs in lipoidal membrane. Since ofloxacin solubility in water is less than diclofenac sodium, therefore its solubility in the membrane is higher than the solubility of diclofenac sodium.

Conclusion

Film forming gels were successfully formulated with different polymers (Na CMC, HPC, HPMC and carbopol). The most appropriate formulation that was compatible with the requirements of providing sufficient adhesion, drug release and flux was that formulated in Na CMC/CP (2:0.3%).

This formulation appears to be promising as an effective base for delivering diclofenac sodium and ofloxacin for treatment and reduction of oral mucositis.

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