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Polyethylene Glycol as New Permeation Enhancer in Thermosensitive Mucoadhesive Hydrogels Containing Hydrophobic Compound for Vaginal Delivery: An *Ex Vivo* Proof of Concept Study

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Abstract: As an excellent alternative, the vaginal route has been widely investigated to deliver different types of drugs. However, the delivery of hydrophobic drugs via conventional preparation is challenging. Here, for the first time, we investigated using PEG as a permeation enhancer in thermosensitive mucoadhesive hydrogels containing Nile red as a model of the hydrophobic compound. Pluronic® F127 (P127) and Pluronic® F68 (P68) were used as thermosensitive agents, and HPMC was used as mucoadhesive agents. The results showed that the concentration of PEG did not change the gelation temperature, mucoadhesive properties, pH, recovery, and rheological behavior of hydrogels. Finally, using PEG could improve the ex vivo permeation and retention profiles of Nile red up to 20-folds and 15-folds, respectively. Following the promising results of this proof of concept study, the application of PEG in improving the vaginal delivery of therapeutic agents should be carried out.

Keywords: vaginal delivery; thermosensitive, mucoadhesive, polyethylene glycol, permeation enhancer.

1. Introduction

Numerous drug delivery approaches with different delivery routes have been developed, considering factors associated with the disease and the preferred outcome for the suitable administration route [1]. Although the vaginal route has been considered an alternative delivery route, this way is self-applied and well-established [2]. Due to the absence of cells with metabolic enzymes, the vaginal route can avoid the first pass effect, generally in the oral route, which permits the utilization of small dosages of drugs [3–7]. Thus, this could potentially circumvent undesired effects occurrence. Importantly, this delivery route has been reported to be effective because of the large surface space of the vaginal area, thick and uniform vascularization, and excellent mucosa permeability [8]. Several therapy purposes have been targeted for the vaginal delivery route, including local infection in the vagina [9], neoplastic

lesions [10], HIV treatment [11, 12], and contraceptive drugs [11, 13–15]. Furthermore, these therapy purposes have been designed for systemic and local pharmacological effects [8, 16].

Recently, various formulations have been commercially available for vaginal delivery routes, namely solutions, creams, gels, ovules, tablets, and capsules. Nevertheless, the effectiveness of these dosage forms in delivering their active substances has been hampered by several limitations, including leakage, short retention time, stability of the drug, and poor release and delivery of active substances in the vaginal area. Additionally, vaginal fluids in the vaginal cavity would naturally remove the formulation applied into the vaginal and, thus, result in the necessity for replicable administration, leading to poor patient adherence [2, 17]. Hydrogels have attracted researchers' attention to overcome these limitations as an alternative approach to vaginal delivery [17]. With mucoadhesive properties, mucoadhesive hydrogels could be a promising approach for this route [18]. However, using hydrogels in semisolid form could make the patients uncomfortable.

Hydrogels based on the combination of thermosensitive and mucoadhesive have been considered for the effective vaginal route. Without losing their mucoadhesive properties, these systems are liquid at room temperature and transform to gel with high viscosity at body temperature [19]. This characteristic could make the formulation spread and coat the mucosa of the vaginal. As previously mentioned, the drugs intended for vaginal routes can be used for topical and systemic purposes in one treatment. Accordingly, it is crucial to ensure that the delivery approach could improve the localization of drugs in the vaginal and the penetrability in the systemic circulation, particularly for hydrophobic drugs. Polyethylene glycol (PEG) has been widely used as a penetration enhancer in transdermal delivery [20]. However, its effectiveness in the vaginal route has not been studied yet. This study used Nile red as a model compound with high lipophilicity and low molecular weight. Nile red was formulated into hydrogels containing Pluronic® and hydroxy propyl methyl cellulose (HPMC) as thermosensitive and mucoadhesive agents. Specifically, the effect of PEG concentration on the physical characteristics, *ex vivo* permeation, and *ex vivo* retention in the vaginal tissues was finally investigated.

2. Materials and Methods

2.1. Preparation of thermosensitive-mucoadhesive hydrogels.

The cold method was applied to prepare the hydrogels [21–25]. In this study, the composition of the formulations was Nile red, Pluronic® F127 (P127), Pluronic® F68 (P68) and HPMC and PEG 600 with the concentrations of 0.5% w/v, 12.5% w/v, 7.5% w/v and 0.4%. Specifically, five different formulations with different concentration of PEG 600 were used, namely 15% (F1), 10% (F2), 7.5% (F3), 5% (F4) and 0% (F4). P127 and P68 were initially dissolved in cold water (5°C). All compounds were dispersed in water and added into Pluronic® solution. The mixtures were mixed and stored at 5°C for 24 h.

2.2. Characterization of hydrogels.

The hydrogels were characterized for their gelation temperature [26], mucoadhesion properties [27], pH, drug recoveries, rheology, *ex vivo* permeation, and *ex vivo* retention in porcine vaginal tissue [18].

3. Results and Discussion

3.1. Preparation of thermosensitive-mucoadhesive hydrogels.

The experiment was a proof of concept study aiming to evaluate the effectiveness of PEG as a permeation enhancer in vaginal preparation. As a model of low molecular weight and hydrophobic drug, Nile red was used as an active compound. The addition of a mucoadhesive agent, HMPC, was done to avoid the normal physiology system of the vagina, which is able to rinse the formulation. Pluronics, well-recognized as Poloxamers, are synthetic triblock copolymers comprising poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO). These polymers are able to show sol-to-gel transition at a specific temperature.

3.2. Characterization of hydrogels.

Following numerous preliminary studies, five final formulations were prepared, as mentioned in Section 2.1. Table 1 depicts the results of this evaluation. The results showed that all formulations were able to form a gel in the vaginal temperature, which is favorable for vaginal administration. It was noted that PEG concentration affected the gelation temperature of the prepared formulation. The increase in PEG concentration decreased the gelation temperature of hydrogels. However, this effect was not significantly different ($p > 0.05$). Importantly, following the dilution using simulated vaginal fluid, the gelation temperature did not change significantly ($p > 0.05$). Accordingly, when applied in the vaginal cavity, the transition to gel after being diluted with simulated vaginal fluid could be anticipated.

Table 1. The gelation temperature of thermosensitive mucoadhesive hydrogel formulations (mean ± S.D., *n=3*).

	$T_{sol-gel}$ (without dilution)	**$T_{sol-gel}$ (with dilution)**
F1	32.91 ± 2.09	33.02 ± 3.21
F2	34.19 ± 3.02	34.43 ± 3.19
F3	34.89 ± 3.18	35.05 ± 3.17
F4	35.95 ± 3.21	36.87 ± 3.28
F5	36.92 ± 3.76	37.22 ± 3.13

The ability of the hydrogels to be attached to the vaginal mucosa is crucial in this study. It was found that the mucoadhesion strength of F1, F2, F3, F4, and F5 were found to be 365.12 ± 31.87 dyne.cm^2, 362.09 ± 30.28 dyne.cm^2, 352.76 ± 37.16 dyne.cm^2, 342.87 ± 32.87 dyne.cm^2 and 337.81 ± 32.09 dyne.cm^2, respectively (Figure 1A). In our preliminary study, without adding HPMC as a mucoadhesion agent, the mucoadhesion strength was observed to be 209.36 ±26.29 dyne.cm^2. Analyzed statistically, the mucoadhesion strength values of F1-F5 were statistically different ($p < 0.05$) compared to the formulation without HPMC. HPMC contains a carboxylic acid group, which is able to form a hydrogen bond with the glycoprotein of mucin, improving the attachment of the formulations containing HPMC [28]. Moreover, the mucoadhesion time evaluation was assessed to predict the residence time of the formulation after being applied in the vaginal cavity. Figure 1 B shows that all formulations possessed mucoadhesion times of > 8 h. Significantly, without HPMC, the mucoadhesion time was less than 3 h. Importantly, despite affecting the mucoadhesion properties of hydrogels, the effect of PEG concentration was considered to be nonsignificant ($p > 0.05$)

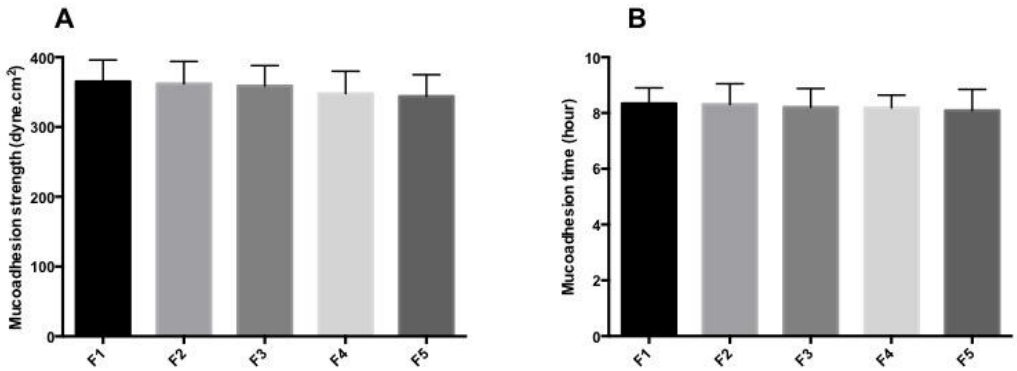

Figure 1. The mucoadhesion strength and time of thermosensitive and mucoadhesive hydrogels (mean ± S.D., *n=3*).

The normal pH of the vaginal environment is 4.5-5.5. The results in Figure 2A depict that all formulations possessed pH in this range. The assessment of viscosity was performed at three different temperatures, as shown in Figure 2B. It was desired that at cold and room temperature, the formulation showed free-flow liquid so that it could easily enable the application while altering into gel at vaginal temperature to improve the residence time. With respect to the rheological behavior, since the hydrogel system generally shows pseudoplastic behavior in liquid and gel forms, the thermosensitive hydrogels system should exhibit shear-thinning behavior. This behavior shows the decrease of viscosity when the shear rate increases. As shown in Figure 2C, all formulations exhibited this behavior. Importantly, in the recovery evaluation, it was found that the recovery values were found to be between 99-100%, indicating the homogeneity of the system.

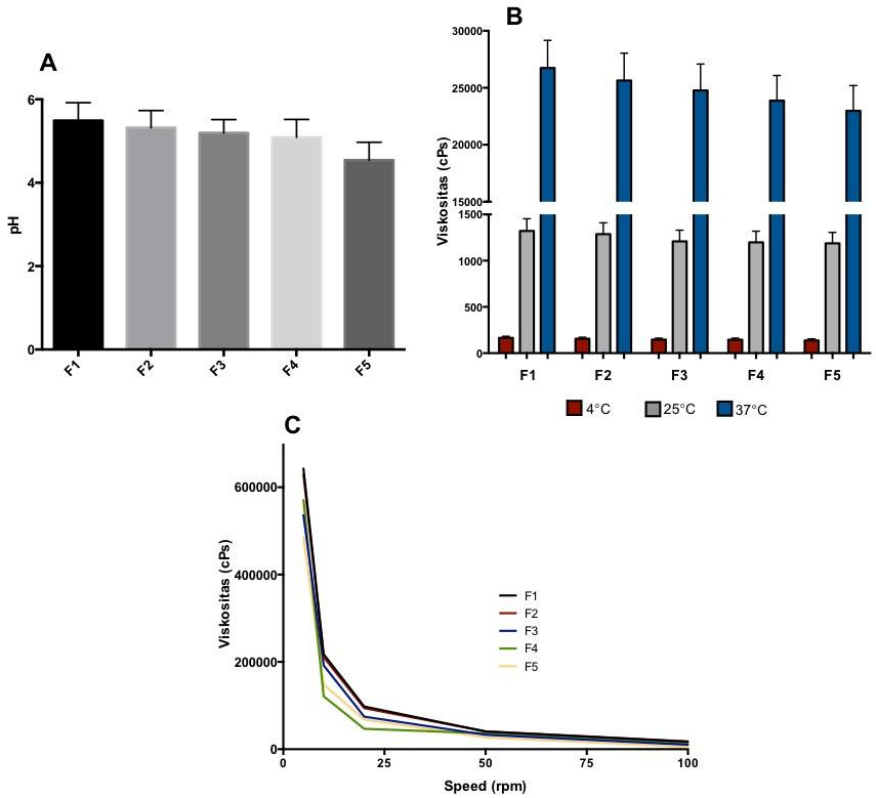

Figure 2. (**A**) pH of the formulations (mean ± S.D., *n=3*). (**B**)Viscosity values of the formulations at 4ºC, 25ºC, and 37ºC (mean ± S.D., *n=3*). (**C**) The rheology behavior of the formulations.

Figure 3A shows the amount of active compound permeated *through* the vaginal tissue over 8 h. Without the use of PEG (F5), due to the hydrophobicity of the compound, only 16.31

± 2.12 μg was detected in the receiver compartment. Importantly, with PEG, the concentrations of compound detected were 327.16 ± 35.18 μg for F1, 309.21 ± 32.77 μg for F2, 217.76 ± 25.31 μg for F3, and 87.28 ± 9.02 μg for F4. All these values were statistically different ($p < 0.05$) when compared to F5. Therefore, using PEG could increase the permeability of hydrophobic compounds *through* vaginal tissue, resulting in the presence of the drug in systemic circulation.

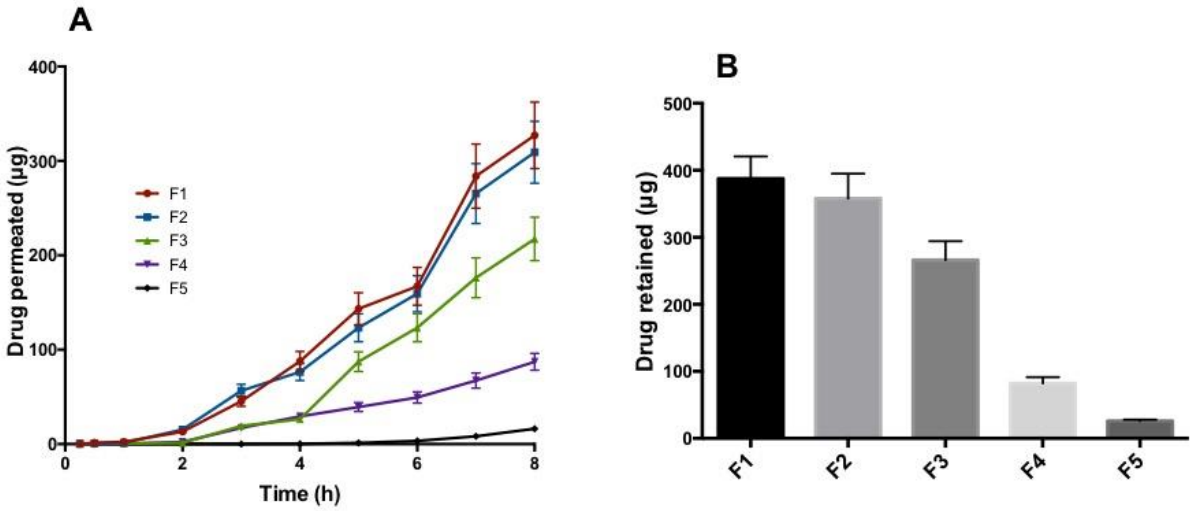

Figure 3. (**A**) The release profiles of ITZ permeation studies using cow vaginal mucosa (mean ± S.D., *n=3*) and (**B**) the amount of ITZ retained in the vaginal mucosa (mean + S.D., *n=3*).

As previously discussed, it is important to have drugs accumulated in the vaginal tissue in some infectious diseases. Figure 3 B shows the compound localized in the vaginal tissue after 8 h after applying the hydrogels. The concentrations of compound retained in the vaginal tissue were 387.61 ± 33.29 μg, 358.11 ± 37.01 μg, 266.17 ± 28.19 μg, 82.18 ± 7.31 μg and 25.87 ± 1.87 μg for F1, F2, F3, F4 and F5, respectively. Like permeation profiles, PEG 600 also improved the localization of hydrophobic compounds in the vaginal tissue. Therefore, this *ex vivo* study shows the potential of the utilization of PEG to improve the concentration of the hydrophobic compound in the systemic circulation and the vaginal. However, further studies incorporating therapeutic agents should be conducted, and *in vivo* studies should be performed.

4. Conclusions

The present work developed a model for thermosensitive mucoadhesive hydrogels containing hydrophobic compounds using Nile red. Specifically, this study investigated the effect of PEG on characteristics and *ex vivo* permeation and retention of Nile red in the vaginal tissue. The results showed that using PEG did not change the properties of thermosensitive mucoadhesive hydrogels. Importantly, for the first time, PEG could be potentially used as a permeation enhancer in vaginal hydrogel preparations, providing improved permeation and retention in the vaginal tissue.

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Conflicts of Interest

The authors declare no conflict of interest.

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