

ORIGINAL ARTICLES

Preparation, characterization and in-vivo evaluation of double-phased mucoadhesive suppositories containing diclofenac in rats

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ABSTRACT

A trial for restriction of drug absorption from suppositories to only the lower part of the rectum, mucoadhesive diclofenac (DC) suppositories were prepared using Witepsol W-25 as a base, carbopol 934 (CP) and white bees wax (Wax) as additives. CP has a mucoadhesive property and wax added to give the suppositories the required stiffness. Double – phased suppositories were prepared as a front layer that contains 10% CP and 20% wax and a terminal layer that contains DC with different ratios of CP. Double – phased and single – phased suppositories containing CP alone nearly exhibit the same in-vitro release profiles, while values of AUC_{0-6h} and MRT of DC after rectal administration of double-phased suppositories to rats were higher than those for single-phased suppositories with or without CP. Moreover, the initial plasma metabolites concentrations after rectal administration of double- phased suppository were significantly lower and tended to exhibit delayed T_{max} compared to single phased suppository. These results proved that the double-phased mucoadhesive suppository could suppress the initial metabolism of DC and may be useful in improving the bioavailability of some drugs as DC.

Key words: double-phased suppository; mucoadhesive; diclofenac; witepsol; carbopol, white bees wax.

Introduction

Conventional suppositories, a polyethylene glycol (PEG)-based suppository, which may soften or melts lately in the rectum due to its relatively high melting point, can not be readily absorbed in the rectal mucous membranes (Burstein *et al.*, 2000; Eboke *et al.*, 1997; Huang *et al.*, 1987 and Nagatomi *et al.*, 1997).

Furthermore, such a PEG – based suppository, which may reach the end of the colon, has a loss of drug at colonic level and may also allow the carried drugs to undergo the first-pass effect (Huang *et al.*, 1987; Choi *et al.*, 1998; Yahagi *et al.*, 2000). To solve the problems of conventional suppositories, it would be desirable to develop double-layered suppositories. Huang *et al.*, 1987 designed double-layered suppositories using Unilubu[®], Hiviswako[®] and polyethylene glycol to aid lower rectal absorption, but their results were not sufficient. Thus, in this study, we attempted to restrict drug absorption from suppositories to the lower rectum. Mucoadhesive double-phased suppositories were prepared using witepsol W25 as a base, carbopol 934 (CP) and white bees wax (wax) as additives. CP is a carboxyvinyl polymer with a mucoadhesive property and has been reported to be mucoadhesive material (Yahagi *et al.*, 2000; Jadhav *et al.*, 2009; Kamel *et al.*, 2006; Keny and Lourenco, 2010). Wax was added to raise the melting point of the base (Tanabe *et al.*, 1985), which helps the suppository to keep its shape and prevents it from melting and extending over a wide area. Transdermal delivery of therapeutic agents is a popular method because mucous membranes are relatively permeable, allowing for rapid uptake of a drug into the systemic circulation and avoiding the first pass metabolism. This efficient uptake offers several benefits over other methods of delivery and allows drugs to circumvent some of the body's natural defense mechanisms (Kellaway and Warren, 1996). To serve as mucoadhesive polymers, the polymers should possess some general physicochemical features such as predominantly anionic hydrophilicity with numerous hydrogen bond-forming groups, suitable surface property for wetting mucous/mucosal tissue surfaces and sufficient flexibility to penetrate the mucous network or tissue cervices (Lenaerts and Gurny, 1990).

Diclofenac was selected as a model drug, since it was applied to rectal suppository form due to its absorption in the rectum (Yong *et al.*, 2005; Schneeweis and Muller-Goymann, 1997) and undergoes first-pass effect (Bort *et al.*, 1999) and plasma concentrations of DC and its metabolites were measured.

Materials and Methods

Materials:

Diclofenac was received as a gift from Novartis Pharma S.A.E, Cairo, Witepsol W25 was supplied by Nobel Dynamitte, Germany. Carbopol 934 (B.F. Goodrich Co., USA). Other materials were of analytical grade and used without further purification.

2.1. Preparation of suppositories:

Witepsol W25 and white wax were melted separately then mixed at about 60 °C, carbopol was added and mixed by ultrasonication (Ultrasonicator, Sonix IV, Model (SS101H), (USA) for about 10 minutes (Yahagi *et al.*, 1999). Diclofenac (100 mg) was then added to the last mixed base and mixed thoroughly then poured into a suppository mold (2g weight) and cooled at room temperature. The same procedures were carried out to prepare suppositories containing DC and carbopol only. Double – phased suppositories were prepared as follows; first, the base mixture for the front layer (Anchoring phase) containing witepsol W25, carbopol 10% and wax 20%, was poured into the mold, cooled, removed from the mold. Then the top of 20 mm of the base (about 1.4 gm) was cut off and used. This front layer was put into the mold again and a terminal layer (drug releasing phase) containing witepsol W25, carbopol with different concentrations; 2, 5 and 10%, respectively, was poured next to the front layer at approximately 60 °C and cooled at room temperature. Suppositories were stored at 10 °C and used within one week.

2.2. Measurement of the hardness of base:

The melted base was poured into a mold and cut into plates of 5x5x20 mm after solidifying. The peak value of the shearing stress at breaking under pressure of 30 cm/min using tooth press stick B was measured at approximately 20 °C using a rheometer (Fudoh Kogyo, Japan) and used to indicate base hardness.

2.3. Measurement of the melting point of the base:

Base melting point was measured by differential scanning calorimeter (Perkin Elmer and Analytical Sciences, Shelton, CT, USA). Base was crushed using mortar and pestle, 5 mg was used as the test sample. Alumina powder (5 mg) was used as a standard sample and measured at a heating speed of 3 °C/min. The temperature of the DSC curve peak was regarded as the melting point of the base.

2.4. Dissolution test:

All the prepared suppositories containing 100 mg diclofenac were inserted into a semipermeable membrane tube. Both sides of the tube were tied up with a thread to prevent leakage. The semipermeable membrane tube was then placed in a dissolution tester (Dissolution apparatus, USP standard, DA-6D, Bombay, India). Dissolution test was performed using the paddle method at 100 rpm, 500 ml phosphate buffer pH 6.8 at 37±0.5°C (JPXIII second fluid) as a dissolution medium (Abdel Hady *et al.*,2003; Chaudhari *et al.*,2009). At 1 hr interval, 5 ml of the medium was sampled and filtered. The filtrate was analyzed by UV – Vis variable wavelength detector (Ultraviolet spectrophotometer, UV-1601 PC Shimadzu, Kyoto, Japan) at 277 nm (Iwata *et al.*, 1999).

2.5. Pharmacokinetic study:

2.5.1. In vivo experiments:

Male Sprague – Dawley rats weighing 250 ± 20 mg were fasted for 24 – 36 hr prior to experiments but allowed free access to water. Eight rats were divided into two groups. The rats in each group were administered with single phased suppositories containing carbopol alone and double-phased suppositories.

Double – phased suppositories with front layer composed of witepsol W25 (W25), carbopol 10% and wax 20% and a terminal releasing layer containing diclofenac (DC), witepsol W25 and different concentrations of carbopol (2%, 5% and 10%), respectively. Suppositories were composed of 2.5% diclofenac.

2.5.2. Administration and blood collecting:

Each rat anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position with a thread. A polyethylene tube was inserted into the right femoral artery of the rat. Suppositories were

administered with a dose which is equivalent to diclofenac 37.5 mg/kg into the rectum 4 cm above the anus (Choi *et al.*, 1998). Half milliliter of blood was collected from the right femoral artery at various intervals and centrifuged at 3000 rpm for 10 min using a centrifuge 4515C (Eppendorf, USA) (Yong *et al.*, 2005 and Schneeweis and Muller-Goymann, 1997).

2.5.3. Blood sample analysis:

Plasma (0.1 ml) was mixed with 0.4 ml of acetonitrile solution containing flufenamic acid (0.5 µg/ml), as an internal standard. It was then centrifuged at 3000 rpm for 10 min to precipitate the proteins. The supernatant layer (0.4 ml) was evaporated under N₂ gas. The residue was reconstituted in 50 µl of ethanol. Then, the resulting solution was analyzed by HPLC (Hitachi, Model L-7100) equipped with an Inertsil ODS-3 C18 column (GL Science, 0.5 µm, 15 cm x 0.46 cm i.d.) and UV detector (Model L-7450). The mobile phase consisted of acetonitrile and phosphate buffer (pH 6.8) (4:6 v/v). The eluent was monitored at 280 nm with a flow rate of 1.0 ml/min (Garcia *et al.*, 1998; Idkaidek *et al.*, 1998; Pinto Pereira *et al.*, 1999).

Results and Discussion

3.1. The hardness of the base:

Considering the practical use of suppositories, the effects of CP and wax on base hardness were examined. Table (1) exhibited that the base hardness was not decreased by addition of CP and wax, suggesting that addition of 10% CP and 10-30% wax to W25 doesn't spoil the mechanical strength of the suppository required for clinical usage.

Table 1: Effect of white beeswax content on suppository base hardness*

Carbopol w/w %	White beeswax	Hardness (Kg)
0	0	1.46 ± 0.48
10	10	1.86 ± 0.17
10	20	2.22 ± 0.09
10	30	1.63 ± 0.40

*Each value represents the mean ± SD of five experiments.

3.2. The melting point of the base:

Endothermic curves of bases containing CP and wax obtained using DSC are represented in Figure (1).

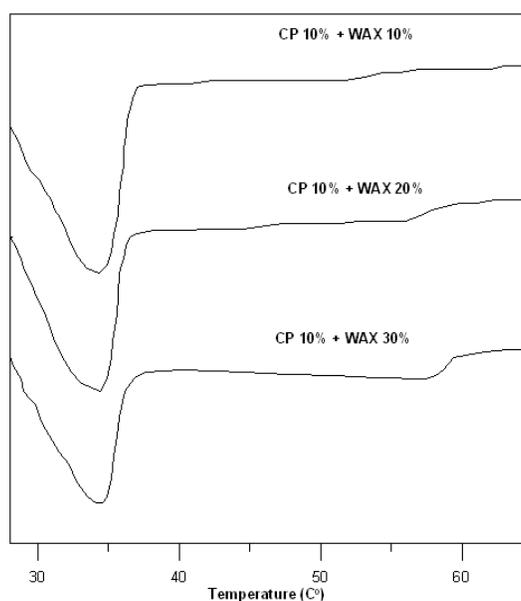


Fig. 1: Endothermic curve for Witepsol W-25 containing 10% carbopol and white beeswax (10, 20 or 30%) in the differential scanning calorimeter heating rate is 3°C/min.

The DSC peak due to fusion of W25 was at about 34 °C. The peak due to fusion of wax appeared at about 58 °C and it became larger with increasing amounts of wax. Addition of wax may raise the melting point of the suppository and enable the suppository to maintain its shape longer. The DSC peak of W25 was not changed by addition of CP and wax. The DSC of CP was not found in the measured range (28 to 100°C), so that the CP dispersed in the suppository should not influence the fusion of the suppository.

3.3. Dissolution of diclofenac from suppositories:

It was reported that addition of wax decreased drug release remarkably (Tanabe *et al.*, 1985). Results of the dissolution test in this study suggested that release of DC from the suppository containing 10% CP and 10% wax at 8 hours was only 10%. These results indicate that addition of wax is not adequate from a viewpoint of drug release. Therefore, a double phased suppositories (Figure 2) composed of mucoadhesive front layer containing 10% CP and 20% wax as the anchoring phase and a terminal layer containing CP and DC as the drug releasing phase were designed. These double-phased suppositories have a larger front layer than the double-layered suppositories used by Huang *et al.*, 1987.

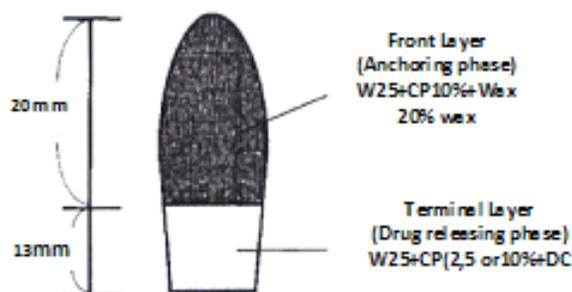


Fig. 2: Structure of double-phased suppository.

Release profiles of DC from double-phased suppositories were not affected by wax in the front layer and they were similar to the single-phased suppositories containing CP alone. Both single- and double-phased suppositories exhibited increased DC release rates with addition of 10% CP (Figure 3). This phenomenon may be due to the water soluble property of CP, i.e. addition of a small amount of CP improved the water absorbability of the base and facilitated the release of lipophilic DC, but large amounts of CP formed a highly viscous gel layer and suppressed the release of DC.

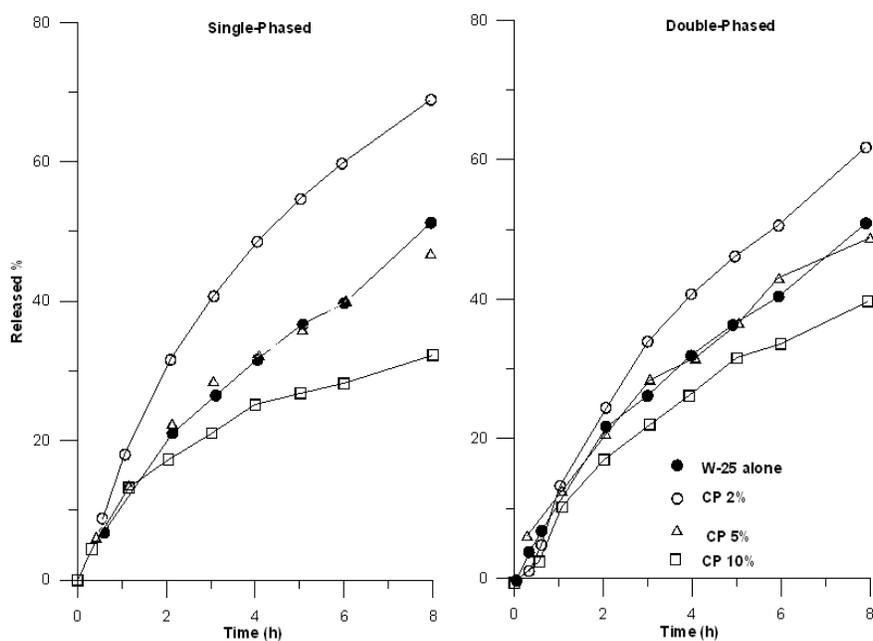


Fig. 3: Release profiles of diclofenac from suppositories in second fluid of JP XIII at 37°C Each point represents the mean of three experiment.

3.4. Animal studies:

Figure 4 (a) shows the plasma concentration profiles of DC after rectal administration of single- and double- phased suppositories to rats. Both suppository types exhibited better absorption compared with W25 suppository, especially the double-phased suppository containing 5% CP, which had the highest C_{max} . Double-phased suppositories had remarkably prolonged plasma concentration profiles compared with single-phased suppositories with a concentration of DC of $10 \mu\text{g/ml}$ at 4 h after administration.

Plasma concentration profile of metabolite, 4' – hydroxyl diclofenac, is shown in figure 4 (b). Diclofenac 100 mg suppository is well absorbed from GIT of six human volunteers and plasma concentrations of 4'-OH DC and DC could be measured while the concentrations of the three other metabolites were below the detection limit of HPLC analysis (Iwamoto *et al.*, 1987). Both single- and double- phased suppositories had T_{max} s that were delayed compared with W25 suppository. The T_{max} delay for double-phased suppositories was especially remarkable and a flat profile was observed for suppositories with 2% CP. These results suggest that double-phased suppositories are retained in the lower rectum, which suppresses metabolism of DC for longer periods and results in high plasma concentration of DC, compared with single-phased suppositories containing CP alone.

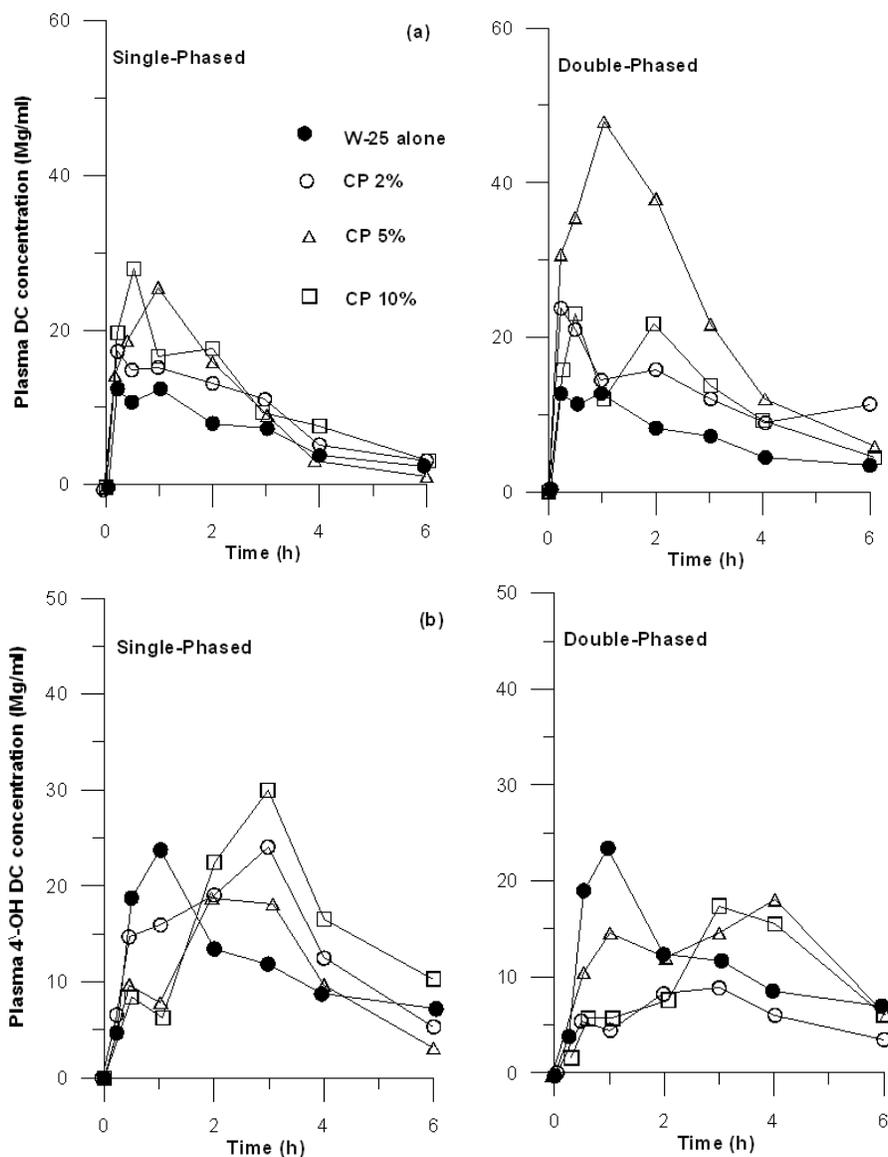


Fig. 4: Plasma concentration profiles of DC (a) 4'-OH DC (b) after rectal administration of rats. Each point represents the mean of three to five rates.

3.5. Pharmacokinetic data analysis:

3.5.1. Single-phased suppositories:

Figure (5) reveals AUC values of DC and AUC ratios of metabolites / DC for different suppositories it has been reported that an optimum concentration of CP exists (Iwamoto *et al.*, 1987) but in this study, AUC of DC became larger with increasing amounts of CP in single-phased suppositories and the AUC ratios of metabolite / DC decreased compared with W25 suppositories. These results suggest that even with addition of CP alone, the absorption region of DC is limited in some extent. However, addition of 2 % CP may be also influenced by an improvement of release rate of DC already described before (section 3.3)

3.5.2. Double-phased suppositories:

On the other hand, in double-phased suppositories, AUC of DC increased the most with the addition of 5 % and exhibited the lowest metabolite / DC AUC ratio as shown in figure (5). These results indicate that the optimum concentration of CP for the drug releasing phase of this double-phased suppository is around 5 %. The absorption region of DC in the double-phased suppository was strictly limited and therefore, the drug release property greatly influenced absorption. Moreover, all MRT (mean residence time) values for DC and metabolite were prolonged for an average of 0.31 h compared with single-phased suppositories containing identical amounts of CP. these results also suggest that the mucoadhesive front layer containing wax prevents movement of the terminal layer containing the drug toward the upper rectum and limits the absorption region of DC to the lower rectum.

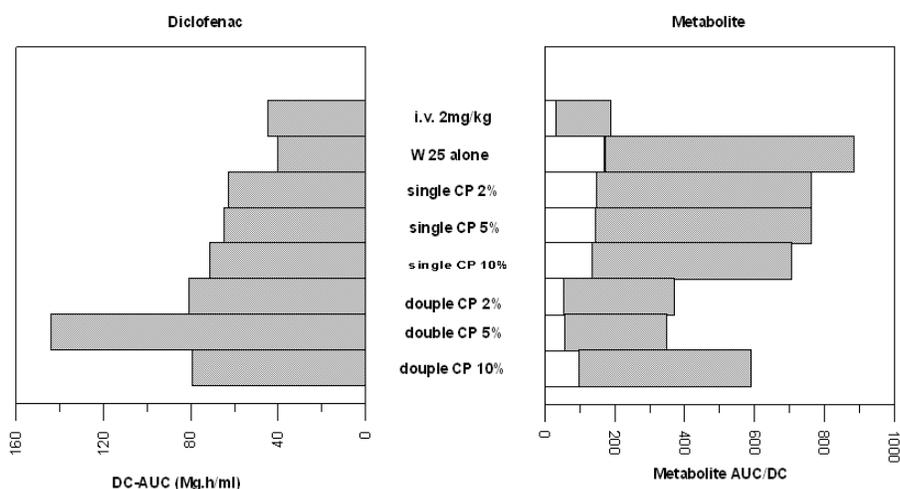


Fig. 5: Comparison of AUCs of diclofenac and metabolite after rectal administration of suppositories to rats. Each point represents the mean of three to five experiments.

4. Conclusion:

The results revealed that that the prepared double-phased suppository with both rectal residence and moderate drug release properties facilitates drug absorption in the lower rectum effectively. This double-phased suppository may be useful for improving bioavailability of drugs with significant first-pass effect like diclofenac.

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