

Preparation and characterization studies of etodolac suppositories: investigation on oleaginous blends of Witepsol® H15

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ABSTRACT: Etodolac is a non-steroidal anti-inflammatory drug that is categorized as a BCS class-II drug due to its low aqueous solubility and high permeability. Frequent dosing, extensive liver metabolism, and low dissolution rates are major limitations of etodolac. The present study focuses on the designing of novel suppository base blends for providing an effective delivery of etodolac. The oils (Caprylic/capric triglyceride, isopropyl isostearate, and isopropyl palmitate) were added to Witepsol® H15 to form suppository base blends. White, odorless, and torpedo-shaped suppositories were developed by using two parts of Witepsol® H15 and one part of the oil. All of the suppositories prepared with different blends were found uniform in weight and content. The mechanical strength of all suppositories was in an acceptable range for suppositories (2.0 - 3.8 kg/cm²). The suppositories showed a disintegration time between 13 and 19 minutes. First-order release kinetics were observed after the *in vitro* release studies. The suppository base blends released 85%-90% of etodolac in the first hour while 60% of etodolac was released from Witepsol® H15 base alone. Prolonged drug release was achieved after 2 hours all of the formulations reached the plateau levels. The oil blends exhibited higher release percentages (96% - 99%) than plain Witepsol® H15 base (89%). Therefore novel suppository base blends could be a promising formulation ingredient for the etodolac suppositories.

KEYWORDS: suppository; etodolac; suppository base; oil; Witepsol® H15.

1. INTRODUCTION

Etodolac (ETO) is a non-steroidal anti-inflammatory drug that is used for the management of rheumatoid arthritis and osteoarthritis to alleviate swelling and pain [1]. According to the biopharmaceutical classification system (BCS), ETO is categorized as a class-II drug substance due to its low solubility and high permeability. It has also been reported that its solubility changes depending on the pH [2]. The recommended ETO application for the chronic pain management of arthritis is between 400 mg/day and 1200 mg/day in divided doses. Additionally, frequent application from 200 mg to 400 mg of the dose is required for the acute pain management of arthritis [3]. Frequent dosing, extensive liver metabolism, and low dissolution rate could be considered major drawbacks of ETO usage during arthritis therapy. Besides these limitations, peptic ulcers and gastrointestinal bleedings were reported as the most common adverse effects of ETO [4, 5].

Changing the route of administration or designing an alternative dosage form could be beneficial to overcome the complications of oral ETO administration. Conventional gel formulations with penetration enhancers were reported to provide an efficient therapy [6]. Besides this, novel drug delivery systems such as nanosuspensions [4, 7], microemulsions [8], vesicular carriers [9, 10], and lipid nanoparticles [11] were designed to provide an efficient ETO delivery via transdermal route. Another option could be the conventional rectal suppositories [12] and *in situ* gelling liquid suppositories [3]. Both of the reports announced that the rectal route of administration could provide a safe systemic delivery.

The rectal route of administration provides several benefits such as minimization of the first-pass effect, controlled or prolonged drug release, and high patient compliance for vomiting or uncooperative subjects [13]. There are also various limitations of this route, such as negative perceptions of patients. On the other hand,

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the type of suppository base, aqueous solubility, and small molecular size of the drug are critical parameters for the formulation [13]. While alternative rectal formulations have been presented (hollow type suppositories or *in situ* gelling systems), conventional rectal suppositories stay the most common rectal dosage form [14].

Rectal suppositories have been developed for years to treat various conditions both locally and systemically. The rectal suppositories include anticonvulsants, anti-inflammatory drugs, sedatives, laxatives, analgesics, anti-emetic, and antibiotic agents that have been studied for the management of such conditions: migraine, fever, or pain relief, epilepsy, constipation relief, hemorrhoids, nausea, and vomiting [15]. On the other hand, vaginal suppositories have been a promising alternative for the drug delivery in recent years. Similar bases and preparation techniques are applied for the preparation of vaginal suppositories as with the rectal suppositories. Novel approaches were reported to deliver new molecules for providing an efficient therapy [16, 17]. In a study, conventional and bioadhesive vaginal suppositories of verdanafil were prepared by using Witepsol® H15 for the boosting *in vitro* fertilization process [16]. In a study, conventional vaginal suppositories of ulinastatin were prepared by using Witepsol®-S55 to prevent premature delivery [17]. Alternatively, suppositories could be used as a secondary carrier system. For example, Witepsol® based vaginal suppositories were used as secondary carrier for the progesterone containing nanoparticles to prolong the release of active substance [18]. Therefore, the development of ETO-loaded suppositories could provide an efficient and safe alternative for inflammatory diseases. A small number of research studies announced for the rectal delivery of ETO [3, 12]. An earlier study has compared the bioavailability and bioequivalence of the oral and rectal formulation of ETO [12]. The more recent work has investigated the safety and efficacy of ETO-loaded thermogelling liquid suppositories [3].

This research aimed to design and characterize suppository bases for the effective delivery of ETO. For this purpose, lipophilic suppository base and various oils were investigated to find optimal formulation. The selection was performed by focusing on the mechanism of *in vitro* release and disintegration studies.

2. RESULTS AND DISCUSSION

2.1. Preparation of the formulations

The release profile of drug substances is directly affected by the type of suppository base. There are three well-known types of suppository bases that include: hydrophilic, lipophilic, and miscellaneous groups [16]. The lipophilic suppository base was considered for the formulation due to the lipophilic nature of etodolac and the prolongation of drug release. Witepsol bases (triglycerides of saturated fatty acids) are one of the largest groups in lipophilic suppository bases. Witepsol® H15's melting range and release characteristics are similar to those of cocoa butter [19].

Caprylic/Capric Triglyceride (TG), Isopropyl Isostearate (IPIS), and Isopropyl Palmitate (IPP) were added to Witepsol® H15 (WH15) base to form a novel type of lipophilic suppository base. Two parts of WH15 and one part of the oil was studied when the increased oil amounts were tried in preliminary studies (data not shown). For each suppository base, a replacement factor was calculated by using Eq.1 in section "4.2. Preparation of the formulations". The replacement factors of ETO were calculated in the WH15, WH15-IPIS, WH15-IPP, and WH15-TG were obtained to be 0.8518, 0.8308, 0.8218, and 0.8316, respectively.

White, odorless, and torpedo-shaped suppositories were developed. The average weight of the suppositories was approximately 2 grams and the length of the suppositories was found as 35mm.

2.2. Uniformity of mass

The weight evaluation of the suppositories was performed by using the compendial method. The average weight of suppositories was around 2 grams. The results were found in between the acceptance criteria described in the pharmacopeia, where non of the suppositories differ by more than 5% [20]. The data were presented in Table 1.

2.3. Uniformity of content

Content uniformity test was performed according to the pharmacopeia, but there is a requirement for an assay to detect the lipophilic active substance in the suppositories. The determination of drug content was accomplished by adapting a previous report [21]. Briefly, individual units from each formulation group were dissolved by using 5 ml of methanol in a 100 ml flask with shaking for 15 minutes. The volume was made up to 100 ml with pH 7.4 phosphate buffer. The solution was then filtered and analyzed by using HPLC.

It was determined that the contents of the suppository formulations containing ETO were in compliance with the limits specified in the pharmacopeia (if the average content of the 10 dosage units is between 90 percent and 110 percent of the content stated on the label) (Table 1). As seen in Table 1, although there was no statistically significant difference in the formulations prepared with Witepsol® H15, it was

determined that the drug content was lower than the other formulations ($p > 0.05$). This is thought to be due to the fact that the oil added to the suppository formulations increases the solubility of the active substance [22, 23].

2.4. Hardness

Hardness or mechanical strength is one of the important critical quality attributes for the suppositories. Both medicated and unmedicated suppositories were found physically robust and the breakpoint of each type of formulation was found to be above 2 kg/cm^2 . In terms of durability and robustness, the literature data also support this finding [14]. Additionally, it was also determined that the mechanical strength of WH15 suppositories was significantly decreased by the addition of the oils ($p < 0.05$). As a consequence, all formulations were evaluated as resistant to forces that may be exposed during transportation and storage.

Table 1. Weight variation, hardness, disintegration time, and drug content of ETO suppository formulations

Formulation code	Weight variation (%)	Hardness (kg/cm^2)	Disintegration time (min)	Drug content (%)
WH15	2.18 ± 0.23	$3.504 \pm 0.103^*$	$17.63 \pm 1.03^*$	-
WH15-IPIS	2.03 ± 0.17	2.719 ± 0.073	14.23 ± 0.59	-
WH15-IPP	2.29 ± 0.41	2.288 ± 0.056	14.84 ± 0.46	-
WH15-TG	1.96 ± 0.08	2.488 ± 0.015	13.78 ± 0.57	-
WH15-ETO	2.32 ± 0.24	$3.775 \pm 0.091^*$	$18.19 \pm 0.72^*$	96.783 ± 2.116
WH15-IPIS-ETO	2.37 ± 0.05	2.915 ± 0.028	14.58 ± 0.66	99.553 ± 1.023
WH15-IPP-ETO	2.15 ± 0.20	2.299 ± 0.018	15.17 ± 1.01	99.358 ± 2.005
WH15-TG-ETO	2.01 ± 0.03	2.517 ± 0.114	13.95 ± 0.23	99.571 ± 1.343

* indicates statistical significance ($p < 0.05$)

2.5. Evaluation of thermal behaviors

Thermal behaviors were investigated by differential scanning calorimetry (DSC). DSC studies were conducted with the active substance, unmedicated, and medicated formulations. The results were shown in Figure 1. The melting point of ETO was determined as $150.1 \text{ }^\circ\text{C}$ which is compatible with the literature data [7]. WH15 exhibited a single endothermic peak with an onset of $33.6 \text{ }^\circ\text{C}$. This value corresponds to the melting point of WH15. Similar thermograms were reported in the literature [24, 25]. Broad endotherms were observed in both unmedicated ($30\text{-}50 \text{ }^\circ\text{C}$) and medicated ($35\text{-}55 \text{ }^\circ\text{C}$) formulations.

The thermal peak of WH15 ($41.2 \text{ }^\circ\text{C}$) shifted to the lower values of temperature with the addition of oils. The peak values were detected as $34.7 \text{ }^\circ\text{C}$, $34.4 \text{ }^\circ\text{C}$, and $35.5 \text{ }^\circ\text{C}$ in unmedicated WH15-IPIS, WH15-IPP, and WH15-TG suppositories, respectively. The addition of an active substance was also causing the alterations in the thermograms. The thermal peak of WH15 shifted to the higher values of temperature with the incorporation of ETO. The peak values were $45.9 \text{ }^\circ\text{C}$, $39.2 \text{ }^\circ\text{C}$, $38.6 \text{ }^\circ\text{C}$, and $38.4 \text{ }^\circ\text{C}$ in medicated WH15-ETO, WH15-IPIS-ETO, WH15-IPP-ETO, and WH15-TG-ETO suppositories, respectively. Similar behaviors (shifting to the higher temperature) were reported for WH15-ibuprofen [24] and WH15-indomethacin [26] mixtures. The incorporation of an active substance does not always elevate the melting point of the suppository base. It was reported that a lipophilic substance such as chloral hydrate depressed the melting point of a suppository base [19].

Furthermore, the onset melting point of the medicated suppositories was evaluated. The onset melting points were found as $37.1 \text{ }^\circ\text{C}$, $35.2 \text{ }^\circ\text{C}$, $35.6 \text{ }^\circ\text{C}$, and $35.4 \text{ }^\circ\text{C}$ WH15-ETO, WH15-IPIS-ETO, WH15-IPP-ETO, and WH15-TG-ETO suppositories, respectively. Thus, the formulations easily melt under normal physiological conditions.

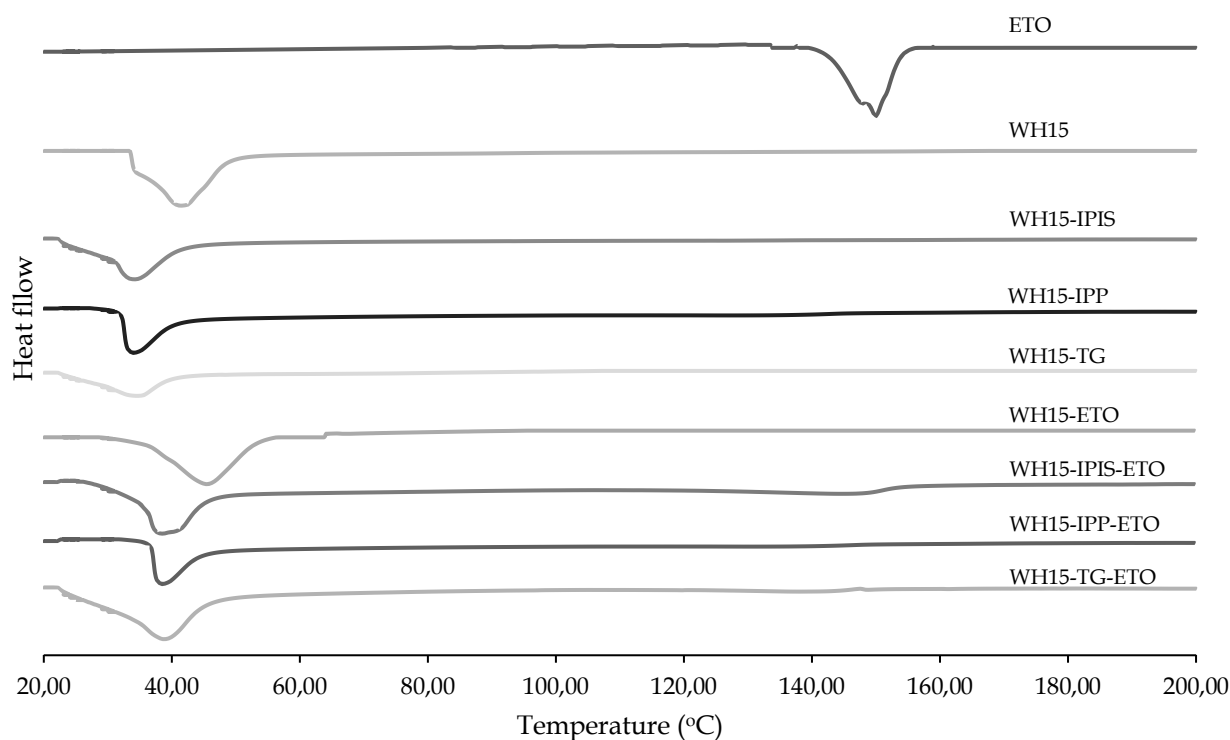


Figure 1. DSC thermograms of active substance and formulations

2.6. Fourier transform infrared (FTIR) analysis

The FTIR spectra of pure ETO, unmedicated, and medicated suppositories are presented in Figure 2. The N-H stretching vibrations of the heterocyclic compounds fall in the region 3500-3000 cm^{-1} [27]. The N-H stretching vibrations have been detected at 3342 cm^{-1} in the FTIR spectrum of ETO (secondary amine group). The C-H stretching vibrations are described in the region 3100-2800 cm^{-1} [27]. The peaks observed at 2873 cm^{-1} , 2924 cm^{-1} , 2970 cm^{-1} , and 3030 cm^{-1} correspond to aromatic C-H stretching in the FTIR spectrum of ETO. The carbonyl C=O vibration is expected to appear in the region of 1750-1600 cm^{-1} [27]. The peak at 1735 cm^{-1} was interpreted as a carbonyl group of carboxylic acid stretching in the FTIR spectrum of ETO. The peaks in the region of 1600-1400 cm^{-1} were interpreted as aromatic ring C-C stretching vibrations as reported in the literature [27]. Thus, the structure of pure ETO was chemically verified.

In the spectrum of WH15, the C-H stretching band of the long fatty acid chain appeared at 2914 cm^{-1} and 2848 cm^{-1} . The carbonyl C=O stretching band was observed at 1726 cm^{-1} and 1738 cm^{-1} . The characteristic peaks in the spectrum of WH15 were in agreement with the literature data [28]. The formation of novel suppository bases was achieved by the addition of oils. The possible interactions between WH15 and IPIS, IPP, or TG were also evaluated. C-H stretching bands of the long fatty acid chain were protected in regions of 2950 cm^{-1} and 2800 cm^{-1} in all types of unmedicated suppository bases. The carbonyl peaks were found between 1750 cm^{-1} and 1700 cm^{-1} for each suppository blend type at decreased % transmittance values. While characteristic functional group peaks were found as protected and the fingerprint region was differentiated. Thus, it was considered excipients chemically compatible with each other.

In the spectrum of medicated suppository bases, the N-H stretching vibrations have been observed at 3310 cm^{-1} , 3338 cm^{-1} , 3308 cm^{-1} , and 3310 cm^{-1} in WH15-ETO, WH15-IPIS-ETO, WH15-IPP-ETO, and WH15-TG-ETO suppositories, respectively. For the same medicated formulation group, it was also observed that the C-H stretching vibrations are found as overlapped position (due to the increase in % transmittance values) in the region 3100-2800 cm^{-1} . The carbonyl vibration in the region of 1750-1600 cm^{-1} was found to be protected. Therefore, the chemical structure of ETO had not changed after the incorporation of suppository bases.

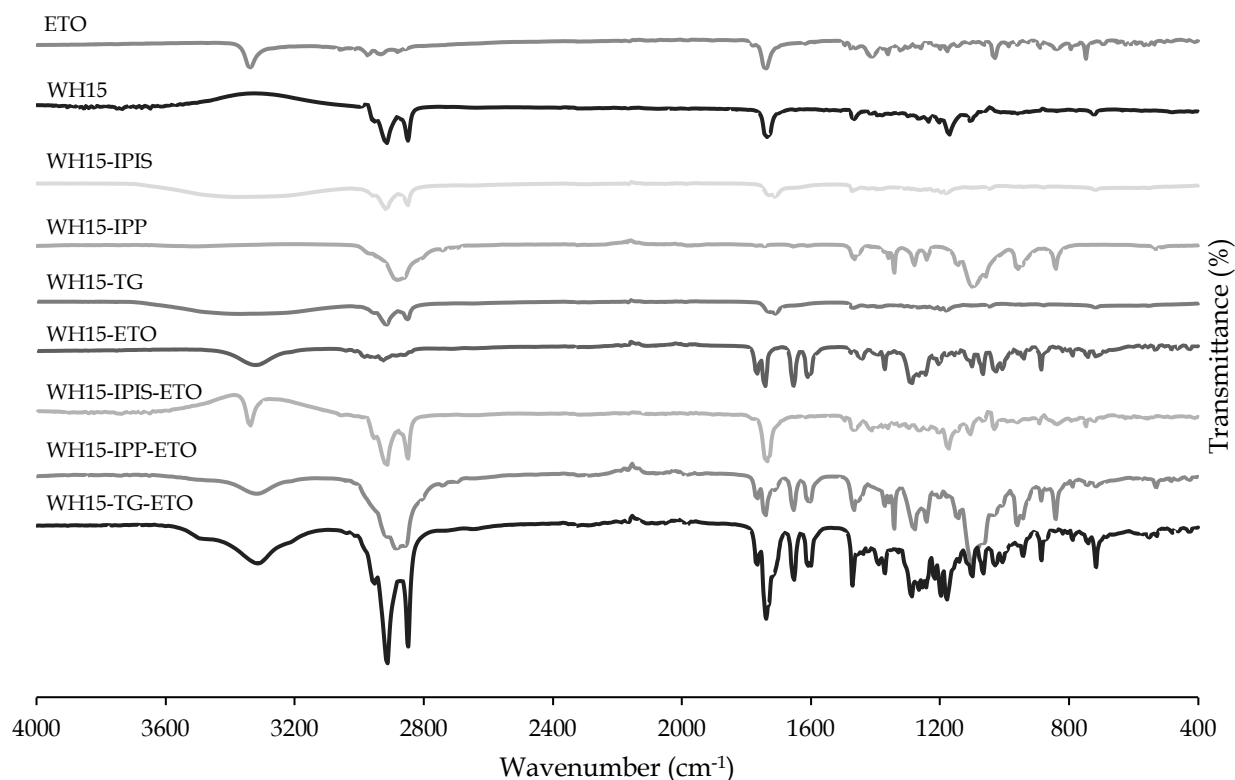


Figure 2. FTIR spectrum of active substance and formulations

2.7. Disintegration time

Disintegration time for suppositories could be defined as the time required for the formulations to soften or disintegrate when placed in the immersion liquid. The formulations showed a disintegration time between 13 and 19 minutes (Table 1). These values are in agreement with the literature and compatible with the compendial data [14, 29].

WH15 suppositories observed significantly lower disintegration when compared to other suppositories ($p < 0.05$). This difference could be explained via thermal interactions. Unmedicated and medicated WH15 suppositories exhibited higher thermal onset values than other blends. Although there was no statistical interaction between other suppository bases, disintegration times were found as correlated with the melting onset values.

2.8. *In vitro* dissolution study

Prior to the dissolution study, compendial and previously reported methods were investigated [2, 14, 30, 31]. Phosphate buffer saline (PBS, pH 7.4) was chosen as the dissolution medium due to representing the rectal pH [14]. The solubility data was reported as 4.5 mg/L in pH 7.4 PBS [2], thus the volume of dissolution medium was adapted (900ml) from the compendial methods [30, 31]. Thus, the sink conditions were provided for the *in vitro* dissolution test of etodolac suppositories.

WH15 suppositories exhibited a remarkably prolonged drug release profile compared with other suppository blends (Figure 3). This outcome was consistent with the observations of the disintegration test. In the first hour of the release study, it was observed that $62.3 \pm 3.2\%$, $86.4 \pm 4.4\%$, $91.2 \pm 2.5\%$, $89.0 \pm 4.1\%$ of ETO released from WH15-ETO, WH15-IPIS-ETO, WH15-IPP-ETO, and WH15-TG-ETO suppositories, respectively. A complete drug release was observed at the end of the second hour include that $89.4 \pm 3.1\%$, $96.5 \pm 4.1\%$, $99.9 \pm 3.7\%$, $99.6 \pm 4.2\%$ of ETO released from WH15-ETO, WH15-IPIS-ETO, WH15-IPP-ETO, and WH15-TG-ETO suppositories, respectively. In a study, conventional and novel (ketoprofen granules prepared with Eudragit RL100) suppositories of ketoprofen were formulated by using different base types [32]. Conventional ketoprofen suppositories prepared with WH15 exhibited remarkably slower release than the conventional suppositories prepared with polyethylene glycol (PEG) bases. In another study, conventional indomethacin and novel (microspheres of indomethacin) suppositories were investigated [33]. It was stated that

conventional indomethacin suppositories prepared with WH15 demonstrated prolonged release when compared to PEG bases. These examples represent the typical lipophilic drug release behavior from oleaginous suppository bases. From this perspective outcomes of the present study are in agreement with the literature.

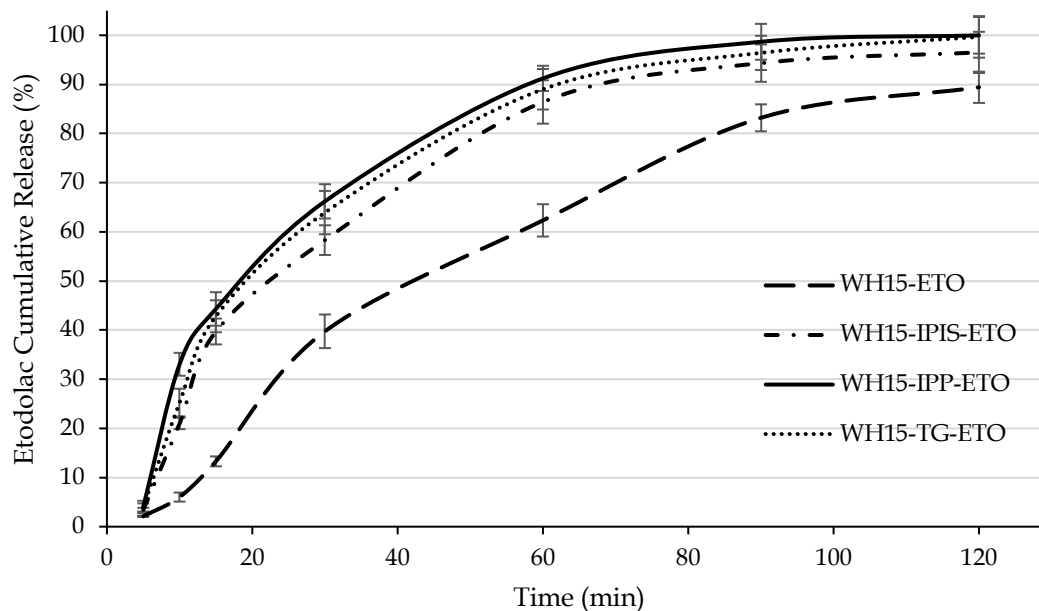


Figure 3. *In vitro* dissolution of suppository formulations

Drug release data were fitted into different mathematical models as seen in Table 2. All of the formulations followed first-order release ($R^2 > 0.91$) that indicated a concentration-dependent drug release. The major release mechanism was diffusion controlled. In a study, tramadol hydrochloride loaded suppositories (prepared with different blends) exhibited a similar release pattern [34].

Table 2. Release kinetics of formulations

Formulation code	Kinetic models					n
	Zero-order R^2	First-order R^2	Higuchi R^2	Hixson-Crowell R^2	Korsmeyer-Peppas R^2	
WH15- ETO	0.866	0.934*	0.801	0.715	0.883	0.213
WH15-IPIS- ETO	0.787	0.941*	0.889	0.758	0.903	0.235
WH15-IPP- ETO	0.759	0.955*	0.895	0.753	0.882	0.247
WH15-TG- ETO	0.781	0.969*	0.883	0.765	0.898	0.301

* indicates statistical significance ($p < 0.05$)

In summary, novel suppository base blends prepared with different oils displayed a significantly higher dissolution profile and release rate compared to the WH15 base alone. Considering the normal physiological conditions, a gradually increased release percent was achieved in two hours.

3. CONCLUSION

In this study, ETO-loaded suppositories were successfully prepared by using several blends of oils. The oleaginous suppository base and several oils were selected for the extension of drug release for efficient delivery. Characterization studies clearly revealed the difference between the WH15 and novel blends. Based on the *in vitro* release studies the impact of oils offers a superior dissolution profile than WH15. Therefore, they could be promising oleaginous suppository base alternatives for an efficient etodolac delivery. It was not possible to make a clear distinction as there was no statistically significant difference between the oils. The outcomes of this research present a baseline for further studies on lipophilic drug substances.

4. MATERIALS AND METHODS

4.1. Materials

ETO (micronized powder) was kindly donated by Nobel Pharmaceuticals (Istanbul, Turkey). Witepsol® H15 was provided by the Sasol GmbH (Witten, Germany). Caprylic/Capric Triglyceride (Crodamol™ GTCC), Isopropyl Isostearate (Crodamol™ IPIS), and Isopropyl Palmitate (Crodamol™ IPP) were obtained from Croda Turkey (Istanbul, Turkey). All chemicals and reagents used in the formulation, production, and analysis stages were of analytical grade.

4.2. Preparation of the formulations

Suppositories were prepared by using the conventional molding technique. Witepsol® H15 and several oil blends were used as suppository bases for the ETO (Table 2). From a pharmaceutical perspective, a 200 mg dose of ETO was selected for each suppository [3, 5]. To prepare suppositories uniformly, a replacement factor is found in which the fraction of the weight displaced part of the base is defined as the number of parts by weight of the drug, by the following equation (Eq.1) [19]:

$$f = \frac{[100(E-G)]}{[(G)(X)]} + 1 \quad (\text{Eq.1})$$

where f is the replacement factor of the drug; E is the weight (gram) of the pure suppository base, G is the weight of suppositories with $X\%$ of the active ingredient, and X is the percent amount (%) of active substance in total weight of the suppository.

The ETO was dissolved in the suppository bases at 60 ± 5 °C and the mixture was poured into the lubricated suppository molds (Josef Uhlmann, Germany) to obtain medicated suppositories. The solidifying process was performed at room temperature for 2h. The medicated suppositories were trimmed, de-molded, and placed into an opaque hermetic container at room temperature. The same production method was applied without ETO to obtain unmedicated suppositories.

Table 2. Composition of suppository bases (% w/w) ^a

Formulation code	IPIS (%)	IPP (%)	TG (%)	WH15 (%)
WH15	-	-	-	100
IPIS	33.40	-	-	66.60
IPP	-	33.40	-	66.60
TG	-	-	33.40	66.60

WH15: Witepsol® H15, IPIS: isopropyl isostearate, IPP: isopropyl palmitate, TG: caprylic triglyceride
^a ETO was fixed at 200 mg for each formulation

4.3. Quantification of ETO

Quantification of ETO was performed by using a high-performance liquid chromatography (HPLC) instrument (1100, Agilent, USA). The previously used HPLC method was slightly changed and validated for the determination of ETO amount [35]. Separation was carried out using a C18 column (250 mm x 4.6 mm, 5 μm) (Thermo Scientific, USA) at 35 ± 1 °C. Acetonitrile: Phosphate buffer (pH 4.5) (60:40, v/v) was used as the mobile phase during the analysis. The flow rate of the mobile phase was 1.8 ml/min. The injection volume was determined as 10 μl and analyzed with a DAD detector (DE3383285, Agilent, USA) at 270 nm. Agilent Chem Station software was used to control the device's components, obtain, and store analysis data.

4.4. Uniformity of mass

The uniformity of mass or weight variation of formulations was performed by using the compendial method [20]. Briefly, twenty suppositories were weighed and the average mass was calculated. The compendial acceptance criterion is limited to 5% of the percentage deviation for suppositories and pessaries.

4.5. Uniformity of content

The uniformity of content was calculated by using the compendial method [36]. This method is based on the assay of the individual contents of the active substance of a number of single-dose units (ten suppositories) to determine whether the individual contents are within limits set with reference to the average content of the sample.

4.6. Hardness

Hardness or breaking force was determined by using a tablet hardness tester (PharmaTest, Germany) at room temperature. As previously reported, the minimum mechanical force was measured to break the suppositories (ten suppositories were tested for a formulation group) [29]. Briefly, each suppository was placed the same way such that the apex was pointed up and the longest side remained parallel with the jaws of the instrument.

4.7. Evaluation of thermal behaviors

Thermal behaviors of suppositories were determined by differential scanning calorimetry (DSC) apparatus (DSC 131, Setaram, France). Previously established method was performed to obtain DSC thermograms [37]. Briefly, 6-8 mg of sample was placed in aluminium pans of the DSC apparatus. The samples were heated in range of 25 to 200 °C with a heating rate of 5 °C/min under a nitrogen flow (20 mL/min). The thermograms were acquired by using SetSoft® software (Setaram, France).

4.8. Fourier transform infrared spectroscopy (FT-IR) analysis

Fourier transform infrared spectroscopy (FT-IR) (iS50 Nicolet, Thermo Scientific, USA) was used to observe the possible chemical interactions of the formulation components. Active substance, medicated, and unmedicated suppository bases were analyzed in range of 4000 cm⁻¹ to 1000 cm⁻¹ in transmission mode. The spectral data was acquired by using Omnic Version 9.0.0 Software.

4.9. Disintegration time

Disintegration time of suppositories was determined by using the compendial disintegration test method [38]. The disintegration test was performed on a basket-rack disintegration test device (PharmaTest Apparatebau, Germany). Distilled water (37 ± 0.2 °C) was used as the immersion fluid and basket moved up and down into the media for 28 – 32 times per minute. The formulations were monitored for complete disintegration during 30 min. The disintegration test was performed as triplicates for each formulation.

4.10. *In vitro* drug release and kinetics

The previously performed compendial method was adapted for the *in vitro* drug release studies [39]. The *in vitro* release profiles of formulations were determined by using a Basket apparatus (Apparatus 1) (PharmaTest Apparatebau, Germany). Suppositories were placed within the rotating baskets (100 rpm) in the 900 mL of phosphate buffer at pH 7.4. The study was conducted at 37±0.5°C. Aliquots (5 mL) were taken from the vessels of the apparatus at predetermined time intervals for 2h. An equivalent volume of fresh medium was replaced at each sampling period. The samples were filtered through a cellulose acetate membrane (0.45 µm). Then, HPLC method was used for the determination of drug content. The results were expressed as mean values (±SD) of drug released percentages at the predetermined time intervals.

Kinetic assessment of *in vitro* release profiles was performed using zero-order, first-order, Higuchi, Hixon-Crowell, and Korsmeyer-Peppas models, as described in equations 2-6, respectively [40].

$$Q_t = Q_0 + k_0t \quad (\text{Eq.2})$$

$$Q_t = Q_\infty (1 - e^{-k_1t}) \quad (\text{Eq.3})$$

$$Q_t = Q_0 + k_H t^{1/2} \quad (\text{Eq.4})$$

$$W_0^{1/3} - W_t^{1/3} = Kt \quad (\text{Eq.5})$$

$$\log [Q_t/Q_\infty] = \log k + n \log t \quad (\text{Eq.6})$$

Q_t and Q_0 , amounts of drug released at time t , and in the release medium at $t = 0$, respectively; k_0 , k_1 and k_H release constants of the zero order, the first order and Higuchi models, respectively; W_0 and W_t , initial and remaining amounts (at time t) of drug in the formulation, respectively; K , constant incorporating the surface-volume relation; Q_t/Q_∞ , fractional release of drug; k and n , kinetic constant and diffusional release exponent indicative of the release mechanism.

4.11. Statistical analysis

Analysis results of the formulations was evaluated by using one-way anova GraphPad software (v.9.13, Prism, USA) ($p < 0.05$).

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