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Research paper

Influence of plasticizers on the stability and release of a prodrug of Δ^9-tetrahydrocannabinol incorporated in poly (ethylene oxide) matrices

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ABSTRACT

The objective of this research was to stabilize a heat-labile novel prodrug of Δ^9-tetrahydrocannabinol (THC), THC-hemiglutarate (THC-HG), in polyethylene oxide (PEO) [PolyOx® WSR N-80 (PEO N-80), MW 200,000 Daltons] polymeric matrix systems produced by hot-melt fabrication for systemic delivery of THC through the oral transmucosal route. For this purpose, the effects of processing conditions (processing temperature and heating duration), plasticizer type and concentration and storage conditions on the stability of the prodrug were investigated. The selected plasticizers studied included vitamin E succinate (VES), acetyltributyl citrate (ATBC), triethyl citrate (TEC), triacetin and polyethylene glycol 8000 (PEG 8000). Furthermore, the influence of plasticizer concentration on drug release was also studied. The stability of THC-HG in PEO matrices was influenced by all the aforementioned variables. Films processed at 110 °C for 7 min were found to be favorable for hot-melt processing with a post-processing drug content of 95%, while significant degradation of THC-HG (~42%) was observed in those processed at 200 °C for 15 min. The degradation of the prodrug during hot-melt fabrication and also upon storage was considerably reduced in the presence of the plasticizers investigated, VES being the most effective. Modulation of the microenvironmental pH to an acidic range via incorporation of citric acid in PEO-plasticizer matrices significantly improved the stability of the prodrug, with almost 90% of the theoretical drug remaining as opposed to only 15% remaining in PEO-only matrices when stored at 40 °C for up to 3 months. The release of drug from PEO matrices was influenced both by the plasticizer type and concentration. A faster release resulted from water-soluble plasticizers, PEG 8000 and triacetin, and with increasing concentration. However, a slower release was observed with an increase in concentration of water-insoluble plasticizers, VES and ATBC.

1. Introduction

Δ^9-Tetrahydrocannabinol (THC), the major pharmacologically active constituent of *Cannabis sativa* has been utilized for the treatment of a variety of clinical conditions. Currently, the most promising clinical indications approved by the Food and Drug Administration (FDA) include treatment of nausea and vomiting in patients requiring cancer chemotherapy as well as for anorexia associated with weight loss in AIDS patients [1]. In addition, THC is reported to have analgesic, anti-inflammatory, anxiolytic, bronchio-dilative, hypotensive, spasmolytic and intraocular pressure reducing activity [2,3]. Despite the promising clinical potential of THC, an effective dosage form has not been developed to date.

Several attempts have been made to deliver THC by various routes of administration. THC has been marketed in USA as Marinol®, a soft gelatin capsule for oral delivery. In this formulation, however, the drug has a limited stability and therefore has to be stored at low temperatures (4 °C) [4]. Moreover, the oral bioavailability of THC is low (~6%) and inconsistent which is mainly due to its high first pass metabolism and poor solubility [5]. In addition, the oral route of drug delivery is not preferable for some severely nauseated patients. The pulmonary route of administration (investigated mostly in the form of marijuana cigarette containing the raw plant material) bypasses first pass metabolism and results in a rapid delivery of THC into the systemic blood circulation due to a large alveolar surface area and the thin epithelium [5]. A bioavailability of 10–35%, related to the amount of THC released from the cigarette, was observed from this route. However, this route is not without significant disadvantages. The effectiveness of a cigarette as a dosage form depends heavily on the smoking process itself. Bioavailability varies according to depth of inhalation, puff duration and

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breath holds [6]. Also, smoking requires elevated temperatures to vaporize and extract which might result in degradation of THC by pyrolysis. In addition, the use of cigarette as a dosage form inherently involves the exposure to various untested marijuana components and other harmful by-products of pyrolysis which may cause bronchial irritation and impaired airway conductance [7].

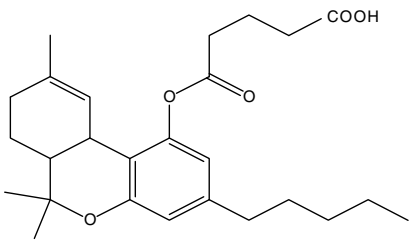
The prodrug approach has been the most successful strategy in improving the pharmaceutical, pharmacokinetic, and pharmacodynamic properties of drugs. Prodrugs are pharmacologically inactive derivatives of active drugs designed to maximize the amount of active drug that reaches its site of action, through manipulation of the physico-chemical, biopharmaceutical or pharmacokinetic properties of the drug. Prodrugs are converted into the active drug within the body through enzymatic or hydrolytic cleavage. To overcome bioavailability problems (due to poor solubility) associated with THC, several prodrugs have been developed and investigated by our research group for affecting the systemic delivery of the drug. These include THC-hemisuccinate (THC-HS), THC-hemiglutarate (THC-HG) and THC-alaninate.

THC-HG, a novel ester prodrug of THC is a viscous liquid that is sticky at room temperature and hardens upon refrigeration. The glass transition temperature is approximately 0 °C indicating that the prodrug is highly unstable when stored at room temperature and hence requires storage under freezing conditions for stability purposes. THC-HG has a molecular weight of 426.53, log P of 3.92 (Moriguchi's method) and is mostly acidic with a pK_a of 3.56 (potentiometric titration). The structure of THC-HG is depicted in Fig. 1.

Due to the significant limitations associated with traditional routes of administration, several non-parenteral routes have also been explored for systemic delivery of THC. These include sublingual [8], rectal [9], nasal [10] and transdermal [11]. With each of these routes having their own disadvantages, an attempt has been made to systemically deliver THC in the form of its novel prodrug, THC-HG through the oral transmucosal route, since it offers unique advantages including avoiding the first pass effect, easy accessibility and enhanced patient compliance.

A survey of the scientific literature indicates that there are only four references on prodrugs of THC (all of them on THC-hemisuccinate) that have focused on studying the stability and bioavailability of the drug in various dosage forms [12–14]. Elsohly et al. have incorporated the hemisuccinate prodrug in lipophilic suppository bases and observed a significantly improved bioavailability (~64%) when administered rectally in dogs [12]. Munjal et al. have investigated the possibility of delivering the hemisuccinate prodrug via oral transmucosal route [14]. However, no research has been focused to investigate the physico-chemical properties or formulation and stability studies of the novel hemiglutarate prodrug in any of the dosage forms to date. Pre-formulation studies conducted by our research group revealed that the hemiglutarate prodrug is highly unstable even when stored at 4 °C [15].

The objective of this work is twofold. Firstly, this work aimed to stabilize the hemiglutarate prodrug of THC via its incorporation into flexible poly (ethylene oxide) (PEO) matrices utilizing a hot-melt method. The effects of processing variables (processing temperature and heating duration), plasticizer type and concentration and storage conditions on the stability of the prodrug were assessed for this purpose. Secondly, the investigation sought to determine and compare the influence of plasticizer types and plasticizer concentration on the release of THC-HG when incorporated in these matrices. These studies are relevant for the overall goal to safely and effectively attain the systemic delivery of THC through the oral transmucosal route utilizing hot-melt extrusion techniques.



Chemical Formula	$C_{26}H_{36}O_5$
Log P	3.92
pKa	3.48
T_g	0.58 °C

Fig. 1. Chemical structure of THC-HG.

2. Materials and methods

2.1. Materials

PEO [PolyOx® WSR N-80 (PEO N-80), MW 200,000 Daltons] was kindly donated by Dow Chemical Company (Midland, MI). Vitamin E succinate (VES), castor oil, ethyl oleate, glyceryl monostearate, diethyl phthalate, isopropyl myristate, glycerol, stearyl alcohol, triethyl citrate (TEC), triacetin and sodium dodecyl sulfate were purchased from Spectrum Chemical, Inc., (Gardena, CA). Acetyltributyl citrate (ATBC), dibutyl sebacate, methanol and acetonitrile (both HPLC grade) were obtained from Fischer Chemicals (Fair Lawn, NJ). Polyethylene glycol 8000 NF (PEG 8000) was procured from Union Carbide Corp., Danbury, CT and glacial acetic acid from J.T. Baker (Phillipsburg, NJ).

2.2. Preparation of polymeric matrices by hot-melt method

Polymeric matrices incorporating THC-HG at 5% w/w were made utilizing a hot-melt method. Briefly, a die containing a 13 mm diameter opening was placed on top of a brass sheet and heated at 110 °C. Approximately 200 mg of physical mixture of drug, polymer and plasticizers/additives was positioned in the orifice of the die, and compressed using a punch. This compressed mixture was heated for 5–10 min to form a melt, followed by cooling under room conditions to form a thin polymeric film. Film thickness ranged from 1.1 mm to 1.3 mm. The diameter of the matrices produced was approximately 12.9 ± 0.2 mm. PEO N-80 grade (molecular weight, 200,000) was used as polymer for all the studies. A polymer:plasticizer:drug ratio of 85:10:5 was used for film fabrication by this method in all of the cases, unless otherwise stated.

2.3. Miscibility of plasticizers with the polymer

The use of a high viscosity polymeric carrier such as PEO for hot-melt processing usually requires the incorporation of a plasticizer into the formulation to lower the polymer melt viscosity, processing temperatures and hence drug degradation. Also, incorporation of plasticizers into a formulation facilitates uniform mixing of the drug with the polymer. For this purpose, various plasticizers were screened for their miscibility with PEO utilizing a hot-melt method at 110 °C. The various plasticizer types studied included vitamin E succinate (VES), castor oil, ethyl oleate, glyceryl monostearate, diethyl phthalate, isopropyl myristate, glycerol, stearyl alcohol, acetyl tributyl citrate (ATBC), PEG 8000, triethyl citrate (TEC), triacetin and dibutyl sebacate. The selection criterion was based on the formation of a film with sufficient flexibility and also on the ease of film fabrication.

2.4. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to further access the miscibility of selected plasticizers with PEO. The plasticizers selected to investigate included, VES, TEC, triacetin, PEG 8000 and ATBC. Approximately 6 mg of polymer-excipient physical mix-

tures (9:1 wt/wt) were weighed and sealed in aluminum pans and then scanned using a Perkin Elmer Pyris-1 DSC instrument. The samples ($n = 3$) were initially subjected to a heat-cool cycle to remove the thermal history of the samples (by heating to 100 °C and holding for 10 min followed by cooling). A second heat cycle was initiated, wherein the samples were heated from 25 to 200 °C. The melting peak of the polymer during the second heating cycle was determined to assess its miscibility with the plasticizer. Nitrogen was used as a purge gas, at the flow rate of 20 mL/min, while the temperature ramp speed was 10 °C/ min for all of the studies.

2.5. Effect of processing variables and plasticizers on post-processing THC-HG stability

THC-HG (5% w/w) was incorporated into PEO matrix systems at four different temperatures (90, 110, 130 and 200 °C), each at a heating duration of 7, 10 and 15 min to investigate the effect of processing variables on the post-processing stability of the prodrug and its extent of conversion to the parent drug during fabrication. The stability of the prodrug was also assessed in the presence of selected plasticizers incorporated into the PEO matrices at two temperatures, 90 and 130 °C. The fabricated matrices were then analyzed via high-performance liquid chromatography (HPLC) method to determine the drug degradation during processing. The matrices were prepared in triplicate.

2.6. Effect of plasticizer type on THC-HG stability

The PEO polymeric matrix systems containing THC-HG (fabricated at 110 °C at a heating duration of 7 min) were stored at four different temperatures of −18, 4, 25 and 40 °C and analyzed at predetermined time intervals for the amount of THC-HG and THC present using a HPLC method. The stability of these matrices were then compared with those containing selected plasticizers (PEO-plasticizer systems) stored at the same temperatures. The results of the stability studies are expressed as a percentage of THC-HG degraded at various time intervals.

2.7. Effect of plasticizer concentration on THC-HG stability

The stability of THC-HG in presence of increasing concentrations (10, 20 and 30% w/w) of five aforementioned plasticizers was investigated by storing the samples at 40 °C and analyzing them at pre-determined time intervals for up to 3 months.

2.8. Microenvironmental pH measurements

Approximately 200 mg of the film was caused to swell and dissolve to form a gel by sonicating it with 1 mL of nanopure water for 30 min. An Orion 710A + pH meter (Thermo Electron Corp., Waltham, MA) was utilized for pH measurements. The pH was recorded by immersing the electrode into the gel matrix and allowing it to equilibrate for 1 min.

2.9. In vitro release studies

The effect of plasticizer type on the release of THC-HG from the PEO matrices were investigated. All the selected plasticizers (10% w/w) were incorporated into the PEO matrices along with the drug utilizing the hot-melt method at 110 C. *In vitro* release studies ($n = 3$) were performed on these matrices utilizing a Hanson SR8-Plus dissolution test system according to USP 31 apparatus 5, paddle over disk method. The polymeric matrix was sandwiched between the watch glass and a mesh so that the release was unidirectional. Nine hundred milliliters of 1% w/v SLS at 37 °C was used as the dissolution medium and the paddle rotation speed was 100 rpm. Samples were collected at pre-determined time intervals and replaced with an equal volume of the fresh dissolution medium. The samples were then filtered through a 0.45 μm nylon syringe filter, and analyzed by HPLC. Also, the effect of selected plasticizers concentration (10, 20 and 30% w/w) on the release of THC-HG was studied.

2.10. Instrumentation and chromatographic analysis

The chromatographic system consisted of a Waters 600 pump and a dual wavelength Waters 2487 UV detector (Waters Corp., Milford, MA). A Luna 5 μ C−18 (2), 150 × 4.60 mm column (Phenomenex, Torrance, CA), was used for the detection of the drug. The mobile phase consisted of 52% methanol, 20% acetonitrile and 18% water with 0.75 mL acetic acid added per 1000 mL solvent. The flow rate was maintained at 1.8 mL/min, with THC-HG and THC eluting at 14 and 10 min, respectively. The injection volume was 20 μL and the column effluent was monitored by UV absorption at 228 nm. The temperature of the column was maintained at 25 °C. A calibration curve was constructed for THC-HG, THC and cannabinol (CBN) using a series of standard solutions of known concentrations, and the area under the peak was employed to determine the concentration of these in the sample solutions. For sample preparation, a weighed portion of the drug-incorporated polymeric matrix was dissolved in a known volume of methanol by sonicating it for 10–15 min. The resulting solution was filtered, transferred into vials and injected into the HPLC system for THC-HG analysis.

2.11. Statistical analysis

Statistical analysis was performed using Microsoft Excel® and a Student's *t*-test was used to analyze the results. The $p < 0.05$ was considered statistically significant.

3. Results and discussion

For oral transmucosal delivery, a flexible polymeric matrix system that adheres to the mucosa for a pre-determined period of time is desirable. Hot-melt processing has been demonstrated to be a viable method for the preparation of drug-incorporated polymeric films [16–18]. Hot-melt processing requires a pharmaceutical grade thermoplastic polymer that can be processed at relatively low temperatures (low melting or glass transition temperatures) due to thermal sensitivity of many drugs. Pre-formulation studies conducted by our research group on THC-HG have revealed that the drug is heat-labile [15]. In this study, THC-HG was incorporated into the hot-melt fabricated PEO matrices intended for systemic delivery of THC through the oral transmucosal route. For fabrication of polymeric matrices by a hot-melt method, a processing temperature of at least 20–50 °C above the melting temperature of semi-crystalline polymer or glass transition temperature of an amorphous polymer is desirable to lower the polymer melt viscosity [19]. Based on our previous studies [15], the lower molecular weight PEO's (PEO N-10 and PEO N-80) allowed the film fabrication at a lower temperature (100–110 °C), while the higher molecular weight PEO's required higher processing temperatures (140–160 °C, based on the molecular weight) to lower their melt viscosity, which resulted in significant degradation of the prodrug. Of the two lower molecular weight PEO's, N-80 exhibited a higher bioadhesion and hence was chosen as the base polymer for fabricating drug-incorporated matrices. Other thermoplastic polymers such as polyvinyl pyrrolidone (PVP), hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC) were also considered, but because of their higher softening

temperatures, degradation at higher temperatures and difficulty in handling, they were determined not to be suitable for hot-melt processing of the prodrug.

3.1. Stability of THC-HG in PEO-only matrices

To assess the stability of THC-HG in PEO-only matrices, drug-polymer matrices were fabricated at 110 °C without any other additives and stored at four pre-determined temperatures, the results of which are presented in Fig. 2. The prodrug was stable for 3 months when stored at freezing condition (−18 °C). Less than 5% degradation was observed at this temperature. However, at the end of 6 months the extent of degradation increased to 10.7%. The drug was very unstable when stored at 4, 25 and 40 °C. THC-HG continued to degrade with time at these temperatures with 55.2% and 20.2% remaining at 4 and 25 °C, respectively, at the end of 6 months. The matrices stored at 40 °C demonstrated complete drug degradation during the same period. An examination of HPLC chromatograms has shown the presence of THC (hydrolytic product), CBN (oxidative degradation product of THC) and other degradant peaks eluting before the prodrug suggesting that several possible degradation mechanisms such as hydrolysis, oxidation and interaction of drug with peroxides present in PEO might be simultaneously playing a role. It is well known that mono- and polyetheric compounds, in particular polyoxyethylene oxides, easily undergo free radical autooxidation during storage and upon exposure to heat, light and transition metals to form peroxides which can be a source of drug degradation by oxidation [20,21]. Polymeric excipients such as PEO, polyethylene glycol and polyvinylpyrrolidone are often produced by polymerization reactions, wherein a high-molecular weight material is prepared and then oxidatively degraded to give the desired molecular weight range [22]. This process leads to the formation of peroxides and other impurities in the polymer which can catalyze the oxidative degradation of the drug. Also, PEO consists of several ether and hydroxyl groups which are capable of potentiating the hydrolysis of THC-HG. These findings clearly demonstrated the instability of this prodrug in PEO matrices.

3.2. Miscibility of plasticizers with polymer

Plasticizers function by weakening the intermolecular forces that hold the polymer chains together and, in doing so, they enhance the ease of movement of polymer chains with respect to each other. These agents lower the glass transition temperature and hence the melt viscosity of the polymer and allow the hot-melt processing to be performed at a lower temperature, thereby improving the processing stability of both the polymer and the drug [19]. The plasticizers utilized for hot-melt processing should be compatible with the polymer with which it is formulated. Compatibility is a property related to a specific polymer/plasticizer system and is a measurement of miscibility of the plasticizer with the polymer [23].

Various plasticizers were incorporated into the PEO matrices to assess their miscibility with the polymer and processed at a temperature of 110 °C by the hot-melt method described previously, the results of which are presented in Table 1. All the plasticizers tested except glyceryl monosterate, dibutyl sebacate, stearyl alcohol and glycerol were found to be miscible with PEO. A few of the plasticizers although miscible with PEO, produced films that were either very brittle/waxy or hard to handle. These included diethyl phthalate, castor oil, isopropyl myristate and ethyl oleate and hence these were not utilized for further studies. Based on the above results, five plasticizers, were selected which included PEG 8000, triacetin, TEC, ATBC and VES.

To further corroborate the results of the miscibility study by the hot-melt method, DSC analysis was performed on the selected plasticizers. The results of the DSC analysis are presented in Fig. 3. The DSC scan of PEO showed a sharp endothermic peak at 70.8 ± 0.2 °C corresponding to its melting point. The melting point of PEO in the presence of VES, PEG 8000, TEC, ATBC and triacetin decreased to 67.9 ± 0.6, 64.4 ± 0.8, 66.3 ± 1.2, 66.8 ± 1.0 and 65.08 ± 0.5 °C, respectively. This decrease in melting point ($p < 0.05$) of PEO clearly indicates the miscibility of these excipients with the polymer [24], which could be of pharmaceutical importance for reducing the degradation of heat-labile drugs such as THC-HG during hot-melt processing.

3.3. Effect of processing variables and plasticizers on post-processing THC-HG stability

A hot-melt method subjects the drug-polymer blends to elevated temperatures for a fixed duration of time, during which the polymer softens and the active ingredient gets either dispersed or dissolved in the molten polymeric matrix if its solubility parameter is similar to that of the polymeric carrier. Hence, the stability of a drug incorporated in a polymeric matrix by hot-melt methods depends on the processing conditions, namely, processing temperature and duration of heating. The results of the influence of processing variables on the post-processing stability of the drug-incorporated PEO matrices are presented in Fig. 4a. The films processed at 90 °C and a heating duration of 7 min exhibited excellent

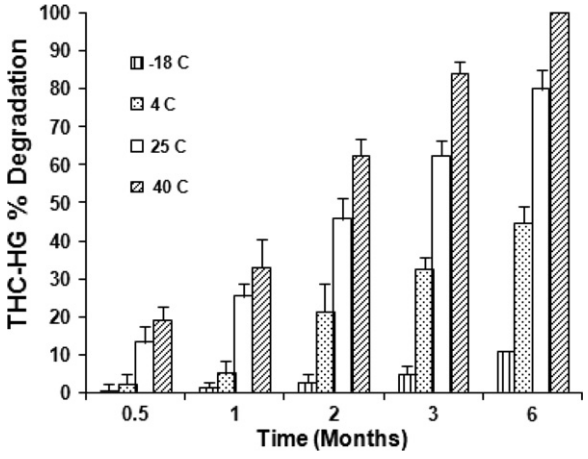
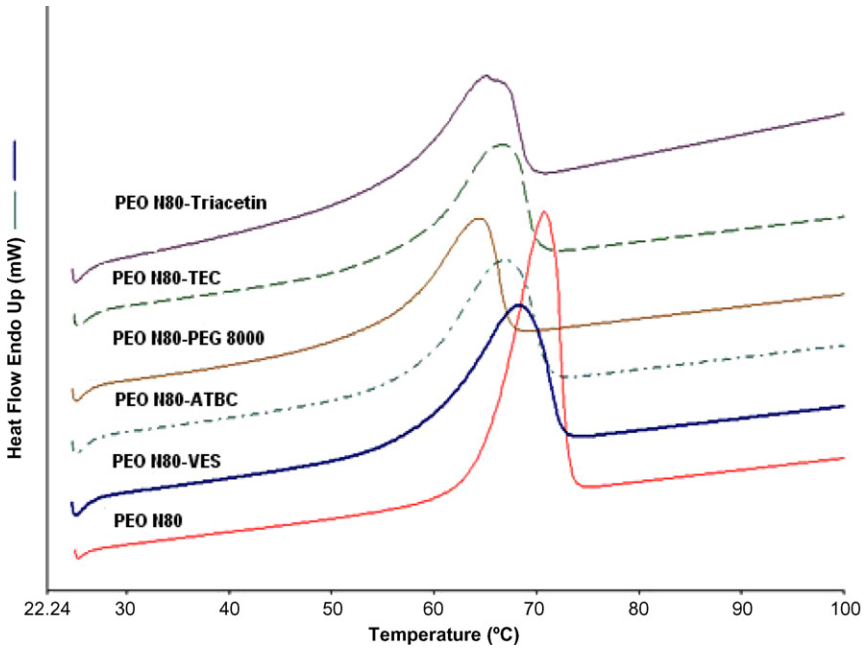


Fig. 2. Stability of THC-HG in the presence of PEO at four different temperatures, −18, 4, 25 and 40 °C. The matrices were prepared by a hot-melt method at 110 °C ($n = 3$).

Table 1
Screening of plasticizers for miscibility with PEO

Plasticizer	Miscibility with PEO
Diethyl phthalate	Miscible; poor film*
Vitamin E succinate	Miscible; good film*
Stearyl alcohol	Immiscible
PEG 8000	Miscible: good film*
Glyceryl monostearate	Immiscible
Isopropyl myristate	Miscible; waxy film*
Acetyl tributyl citrate	Miscible: good film*
Glycerol	Immiscible
Triethyl citrate	Miscible: flexible film*
Triacetin	Miscible: good film*
Ethyl oleate	Miscible; poor film*
Dibutyl sebacate	Immiscible
Castor oil	Miscible; difficult to handle*

* The terminology "good film/flexible film/waxy film/poor film" indicate the handling characteristics of the polymeric matrices and is presently used only for selection of plasticizers [14].



Sample	Melting peak (°C)
PEO N80	70.79
PEO-TEC	66.30
PEO-ATBC	66.85
PEO-Triacetin	65.08
PEO-VES	67.94
PEO-PEG 8000	64.41

Fig. 3. Screening of selected PEO-plasticizer (8:1) physical mixtures by DSC.

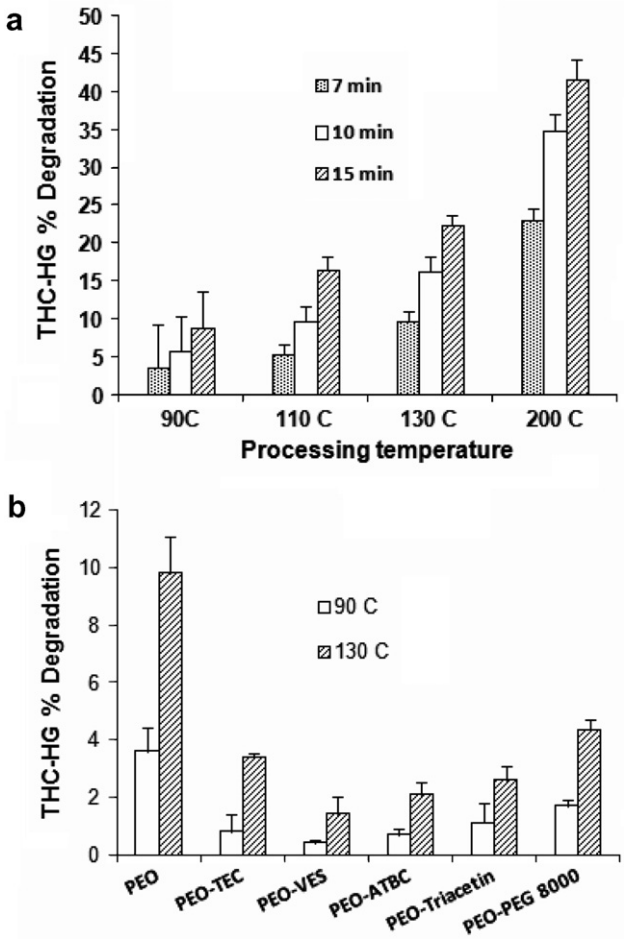


Fig. 4. Effect of (a) processing variables (processing temperature and heating duration) and (b) plasticizers (the films were processed at 90 and 130 °C, each at a heating duration of 7 min), on the post-processing stability of THC-HG in PEO matrices ($n = 3$).

post-processing content with <5% degradation. However, content uniformity of these films was very poor. These films exhibited 3.6%, 6.1% and 9.0% degradation, respectively, at heating durations of 7, 10 and 15 min. Films processed at 110 °C at a heating duration of 7 min exhibited 5.4% degradation, which increased to 9.9% and 16.5% as the heating time increased to 10 and 15 min, respectively. Unlike films processed at 90 °C, the films processed at 110 °C demonstrated good content uniformity at all the heating durations investigated. In contrast, higher processing temperatures (130 and 200 °C) produced significant degradation of the prodrug, which increased with increasing processing temperatures even when exposed to lower heating durations. For example, films processed at 130 and 200 °C at 7, 10 and 15 min exhibited 9.7%, 16.2% and 22.4% and 23.1%, 33.8% and 41.6%, respectively (Fig. 4a). However, the films processed at higher processing temperatures exhibited excellent content uniformity and also were easier to process. These findings may be explained by the fact that higher processing temperatures and heating durations lowered the polymer melt viscosity, allowed the polymer to soften and thereby facilitated the uniform mixing/dispersion of drug in the matrix. Thus, film fabrication was easier at these conditions leading to a uniformity of content. However, these conditions exposed the polymer and the active ingredient to thermal stress leading to their degradation. Based on these findings, a processing temperature of 110 °C and a heating duration of 7 min were found to be favorable for hot-melt processing of drug-incorporated polymeric matrices.

To test the fact that plasticizers lower the melt viscosity and thereby facilitate hot-melt processing of drugs, the polymeric matrices were fabricated incorporating the selected plasticizers (10% w/w) at 90 and 130 °C, each at a heating duration of 7 min, the results of which are depicted in Fig. 4b. Incorporation of plasticizers reduced the degradation of the active significantly as compared to PEO-only matrices with no additives even when processed at a higher temperature (130 °C). For example, the degradation of drug in presence of triacetin, PEG 8000, TEC, VES and ATBC at 90 and 130 °C was found to be 1.1%, 1.7%, 0.8%, 0.4% and 0.7%, respectively, and 2.6%, 4.3%, 3.4%, 1.4% and 2.1%, respectively as compared to 3.6% and 9.8%, respectively, in PEO-only matrices. Also, the films processed in presence of plasticizers were easy to fabricate and exhibited excellent

content uniformity. These findings demonstrated that the processing variables and formulation additives such as plasticizers influence the stability of an active incorporated in hot-melt polymeric matrices and therefore can be modulated to minimize the drug degradation during processing. These results were very positive for hot-melt extrusion (HME) of this prodrug (a subject of our future studies), since the decreased processing time, precise control of heat and uniform mixing facilitated by the rotating screw by this technique will lead to an increased content uniformity, reduced degradation of both active and polymer (PEO) and increased post-processing yield [25].

3.4. Effect of plasticizer type on THC-HG stability

Pre-formulation studies conducted on THC-HG by Thumma and co-workers have revealed that the drug decomposes readily when exposed to heat and air [15]. Due to the amorphous nature and low glass transition (close to 0 °C), the drug has high reactivity even when stored at 4 °C. Since the stability of an active incorporated in any dosage form is largely influenced by the storage conditions, it was imperative to conduct the stability studies at different storage conditions in order to gain an insight into the degradation rate of the prodrug in these matrices.

To assess the influence of selected plasticizers on the stability of the prodrug in PEO polymeric matrices, the fabricated matrices were stored at four different temperatures of −18, 4, 25 and 40 °C, the results of which are depicted in Fig. 5. THC-HG was found to be stable in all the plasticizer-incorporated PEO matrices when stored at −18 °C (Fig. 5a). The degradation was observed to be <5% in these matrices as compared to PEO-only matrices (10.7% degradation) at the end of 6 months. A plot of Ln (% drug degradation) versus time was made and a linear relationship (R^2 ~1.0) was obtained indicating that the THC-HG degradation in these matrices occurs by first order. The degradation rate constants (k) calculated utilizing Eq. (1) are presented in Table 2.

$$k = \frac{\mathrm{Ln}\left(\frac{C_0}{C}\right)}{t}, \quad (1)$$

where 'C_0' is the initial concentration of the drug.

'C' is the concentration remaining undecomposed at time 't'.

The degradation rate constants were lowered in the presence of plasticizers with VES showing the lowest value. The results of drug

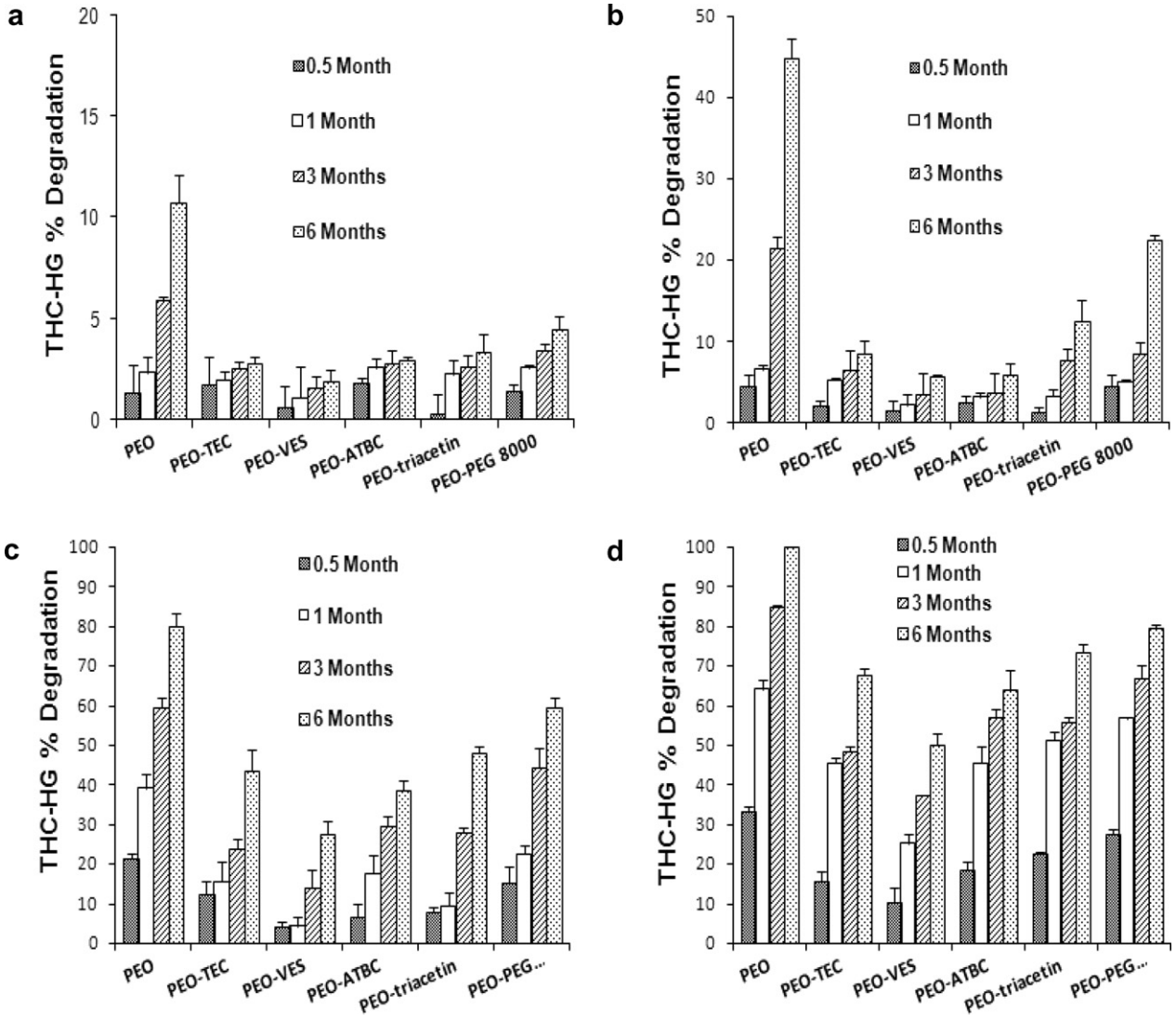


Fig. 5. Effect of plasticizers on the chemical stability of THC-HG in PEO polymeric matrices (n = 3) stored at four different temperatures: (a) −18 °C, (b) 4 °C, (c) 25 °C and (d) 40 °C. The films were fabricated at 110 °C for 7 min.

Table 2
First-order degradation rate constants, k (wk^{-1}) of THC-HG in various PEO-plasticizer systems as a function of temperature

Polymeric matrices with THC-HG	Storage temperature			
	−18 °C	4 °C	25 °C	40 °C
First-order degradation rate constants, wk^{-1}				
PEO N-80	0.0047	0.0248	0.0668	0.2301
PEO-TEC	0.0012	0.0037	0.0238	0.0510
PEO-VES	0.0008	0.0024	0.0133	0.0288
PEO-ATBC	0.0013	0.0025	0.0204	0.0427
PEO-triacetin	0.0014	0.0056	0.0273	0.0551
PEO-PEG 8000	0.0019	0.0106	0.0377	0.0661

stability in the presence of all the selected plasticizers when stored at 4 °C are shown in Fig. 5b. THC-HG was relatively stable in the PEO matrices containing VES, ATBC and TEC (~5% degradation) as compared to PEO-only matrices which exhibited 44.9% degradation at the end of 6 months. However, in PEO-triacetin and PEO-PEG 8000 systems, the drug exhibited 12.6 and 22.5% degradation, respectively, during the same period.

The drug degradation was higher in all the plasticizer-containing matrices (Fig. 5c) at the end of 6 months of storage at 25 °C, as evidenced by the higher '*k*' values observed in these systems (when compared to those at 4 °C). However, this degradation was much lower than that observed with PEO-only matrices (79.9%) as evidenced by the lower '*k*' values in the PEO-plasticizer systems during the same period. Similar results were obtained from PEO-plasticizer matrices stored at 40 °C (Fig. 5d). Complete degradation (100%) of the drug was seen in PEO-only matrices at 6 months, while the PEO-plasticizer systems reduced the degradation of THC-HG by 20–50% (depending on the plasticizer utilized) during the same period. Among all the PEO-plasticizer systems investigated, degradation of the drug was highest in the PEO-PEG 8000 (79.5%) and PEO-triacetin (73.3%), respectively, and lowest in the PEO-VES system (49.9%).

The degradation of drug in the various excipients investigated was found to be on the order of PEO > PEO-PEG 8000 > PEO-triacetin > PEO-TEC > PEO-ATBC > PEO-VES. An examination of the hydrophilic/ lipophilic properties of these excipients reveals that PEO, PEG 8000, triacetin and TEC are hydrophilic, while VES and ATBC are lipophilic in nature. The results of this study indicate that the drug degraded more in a hydrophilic environment and was relatively stable in presence of lipophilic excipients. These findings were consistent with those obtained by Munjal [14] and ElSohly [12] who also reported significant degradation of the hemisuccinate prodrug of THC in the presence of hydrophilic excipients.

3.5. Effect of plasticizer concentration on THC-HG stability

The results of the stability of THC-HG in PEO matrices as a function of plasticizer concentration are presented in Fig. 6. The degradation of the drug in the PEO matrices was found to increase with an increase in concentration of the hydrophilic excipients, PEG 8000 and triacetin (Fig. 6a). For example, the drug degraded 76.3% and 65.6% in PEG 8000 and triacetin, respectively, when employed at a concentration of 30% w/w as opposed to 66.8% and 54.8%, respectively, at 10% w/w. On the contrary, an increase in concentration of lipophilic plasticizers (ATBC and VES) from 10% w/w to 30% w/w led to a reduction in degradation of THC-HG (Fig. 6b). However, in the case of the PEO-VES system, the reduction was seen only until 20% w/w concentration with no significant improvement at 30% w/w. These findings corroborated our previous observation that the drug is unstable in the presence of hydrophilic excipients.

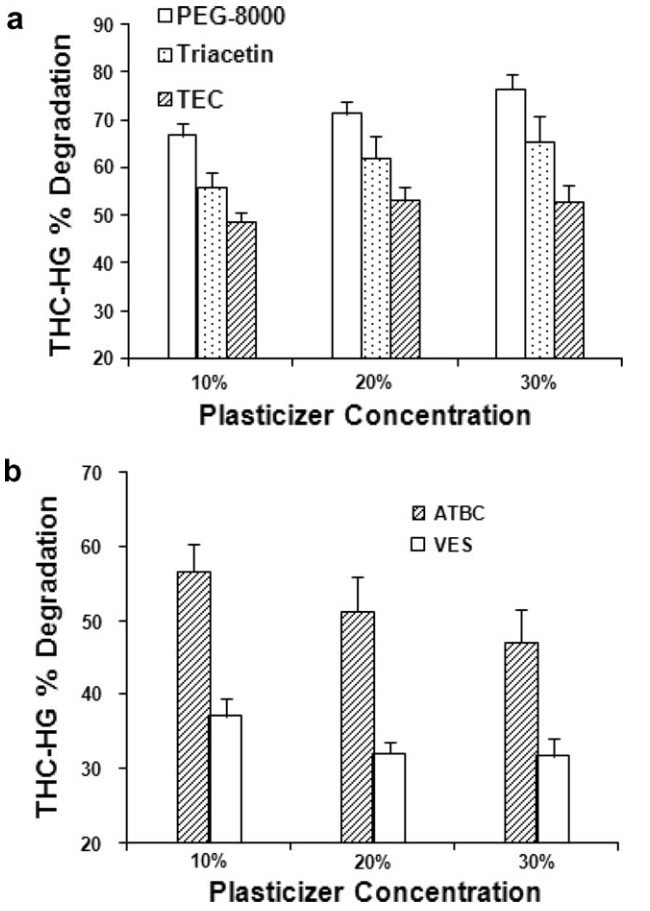


Fig. 6. Effect of (a) PEG 8000, triacetin and TEC concentration, (b) VES and ATBC concentration, on the stability of THC-HG in PEO matrices (at the end 3 months at 40 °C). The films (n = 3) were fabricated at 110 °C for 7 min.

3.6. Influence of citric acid on THC-HG stability

The degradation rate of many active compounds is affected by the microenvironmental pH to which they are exposed in solid dosage forms. Studies on enhancing the stability of drugs by incorporating pH modifiers into the formulation have been reported. The stability of acetyl salicylic acid in a tablet formulation was enhanced by the addition of acidic agents [26]. The rate of hydrolysis of an ester prodrug, DMP-754, was decreased by modulating the microenvironmental pH to ~4.0, in the solid-state [27] .

To assess the role of pH on the stability of THC-HG in the presence of selected plasticizers, pH measurements were made on the polymeric matrices, the results of which are presented in Table 3. It was observed that the relatively stable system (PEO-VES) exhibited a pH of 5.0, corresponding to a relatively acidic environment, but the drug-incorporated PEO and PEO-PEG 8000 matrices exhibited basic pH values. Also, preliminary studies conducted by our research group to investigate the effect of various pH modifiers on the stability of THC-HG found that the drug was stable in the presence of citric acid, while it exhibited significant degradation with basic pH modifiers (unpublished results). These findings suggest that THC-HG is more stable in an acidic environment.

Based on these observations, an attempt was made to further stabilize the drug in PEO-plasticizer systems by the incorporation of citric acid as an acidic pH modifier. For this purpose, drug-incorporated polymeric matrices were fabricated in the presence of citric acid by the hot-melt method at 110 °C. A PEO:plasticizer:drug:citric acid ratio of 82.5:10:5:2.5 was used for the fabrication of these matrices. The films were stored at 40 °C and analyzed at pre-determined time

Table 3
Microenvironmental pH values of the polymeric film formulations containing THC-HG

Matrix formulation	pH
PEO N-80	7.7
PEO-VES	5.0
PEO-PEG 8000	7.5
PEO-ATBC	7.1
PEO-TEC	7.2
PEO-triacetin	7.6
PEO-VES-citric acid	3.1
PEO-PEG 8000-citric acid	2.7
PEO-ATBC-citric acid	3.3
PEO-TEC-citric acid	3.4
PEO-triacetin-citric acid	2.9

intervals for up to 3 months, the results of which are illustrated in Fig. 7. Incorporation of citric acid reduced the microenvironmental pH to ~3.0–3.5 (Table 3) and stabilized the drug in all these matrices. This corresponded well with the pH-rate profile of THC-HG in solution showing a maximum stability in the range 3.0–4.0 [15]. Less than 10% of drug degraded in VES, ATBC and TEC matrices containing citric acid as compared with 36–58% degradation observed in these matrices with no citric acid added at the end of 3 months at 40 °C. PEO-PEG 8000 and PEO-triacetin matrices containing citric acid exhibited higher degradation values (~15%) as compared to the other three matrices investigated. This may be attributed to the pH values in these matrices (2.7–2.9), deviating slightly from the maximum pH stability range of 3.0–4.0. Thus the approach of microenvironmental pH modulation was successfully utilized to stabilize the hemiglutarate prodrug in PEO matrices for the goal of future *in-vivo* studies for oral transmucosal delivery.

3.7. In vitro release studies

Plasticizers incorporated into the pharmaceutical polymers not only facilitate the thermal processing of polymers but also modify the mechanical properties, water absorption behavior, and adhesive property of the polymeric films [28]. All these properties affect the strength and integrity of the polymeric film, which further affect drug release performance. Based on the type and amount utilized, plasticizers can either increase, decrease or not influence the dissolution of drugs incorporated in polymeric films. This difference could be a result of solubility and affinity of the plasticizer to the polymer.

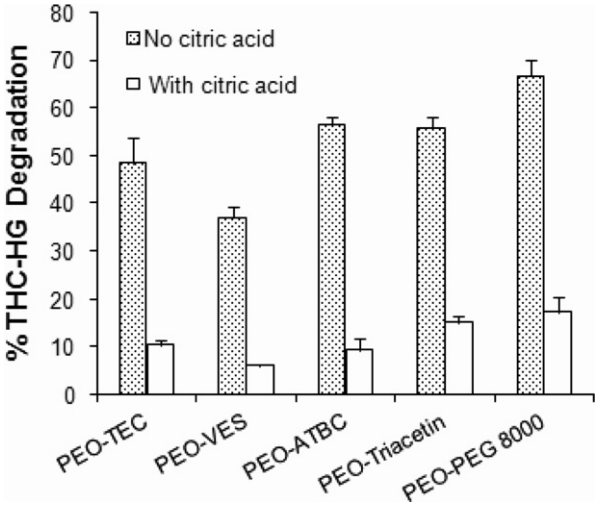


Fig. 7. Stability of THC-HG in PEO-plasticizer matrices in the presence of citric acid anhydrous (at the end of 3 months at 40 °C). The matrices ($n = 3$) were fabricated at 110 °C for 7 min.

This study is aimed to understand how the selected plasticizers influence the release of THC-HG from PEO matrices. A comparison of dissolution profiles was performed using a model independent method [29]. The results of the plasticizer type on THC-HG release are presented in Fig 8. The release rate of the drug from the polymeric film containing PEG 8000 (similarity factor, $f_2 = 35.5$) and triacetin ($f_2 = 43.9$) was faster as compared to the PEO-only matrix with no plasticizer added. TEC matrices exhibited a similar release profile as the PEO-only matrix ($f_2 = 67.7$), while the release of the drug was lowered in the presence of plasticizers, VES ($f_2 = 34.6$) and ATBC ($f_2 = 46.7$). A close examination of the solubilities of these plasticizers indicates that PEG 8000 and triacetin are soluble in water, whereas VES and ATBC are insoluble in water. These results suggest that water-soluble plasticizers might aid the release of drug more than plasticizers with low aqueous solubility. This phenomenon may be explained by the fact that when the release medium diffuses into the polymeric matrix, water-soluble plasticizers such as PEG 8000 and triacetin do not interfere with the movement of water molecules within the matrix. However, water-insoluble plasticizers, such as VES and ATBC, may suppress the aqueous release medium from diffusing into the polymeric matrix [30].

The effects of plasticizer concentration on the release of THC-HG from PEO matrices are presented in Fig. 9. Faster dissolution of the drug resulted when PEG 8000 was used as a plasticizer, with the release becoming faster with increasing plasticizer concentration (Fig. 9a). Similar results were observed with an increasing concentration of triacetin (data not shown). The increase in release rate from polymeric matrices containing water-soluble plasticizers can be an effect of the enhancement of solubility of the drug in the matrix and/or diffusivity [31]. The more water-soluble plasticizers would create channels, allowing more rapid penetration of the dissolution medium into the polymeric matrix and this process is enhanced as the concentration of these plasticizers increase.

The influence of TEC levels on the drug release rate from PEO polymeric matrices is illustrated in Fig. 9b. An increase in concentration of TEC in the PEO polymeric matrices did not influence the drug release. The f_2 values of the formulations incorporated with 20% and 30% TEC were found to be 76.6 and 73.9, respectively, indicating that these dissolution profiles are similar. The release of THC-HG from PEO matrices as a function of VES concentration is shown in Fig. 9c. A slower release rate was observed when VES was used as a plasticizer, with the release becoming slower with increasing plasticizer concentration. Similar results were observed when ATBC was used as a plasticizer (data not shown). These results can be explained by the fact that as the concentration of these

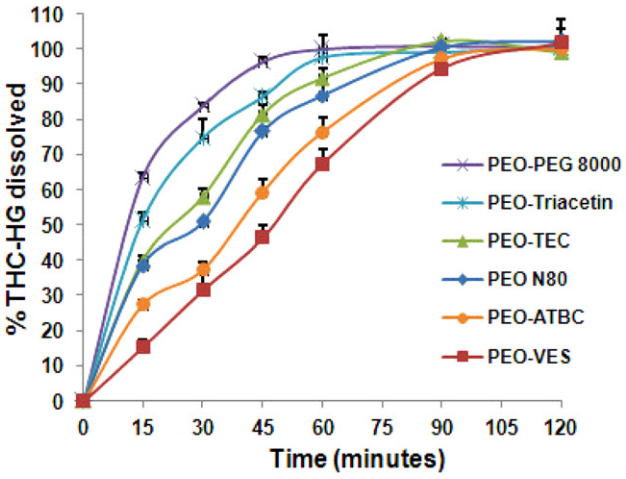


Fig. 8. Effect of plasticizers on the release of THC-HG from PEO matrices ($n = 3$). The films were fabricated at 110 °C for 7 min (PEO: plasticizer: THC-HG – 85:10:5).

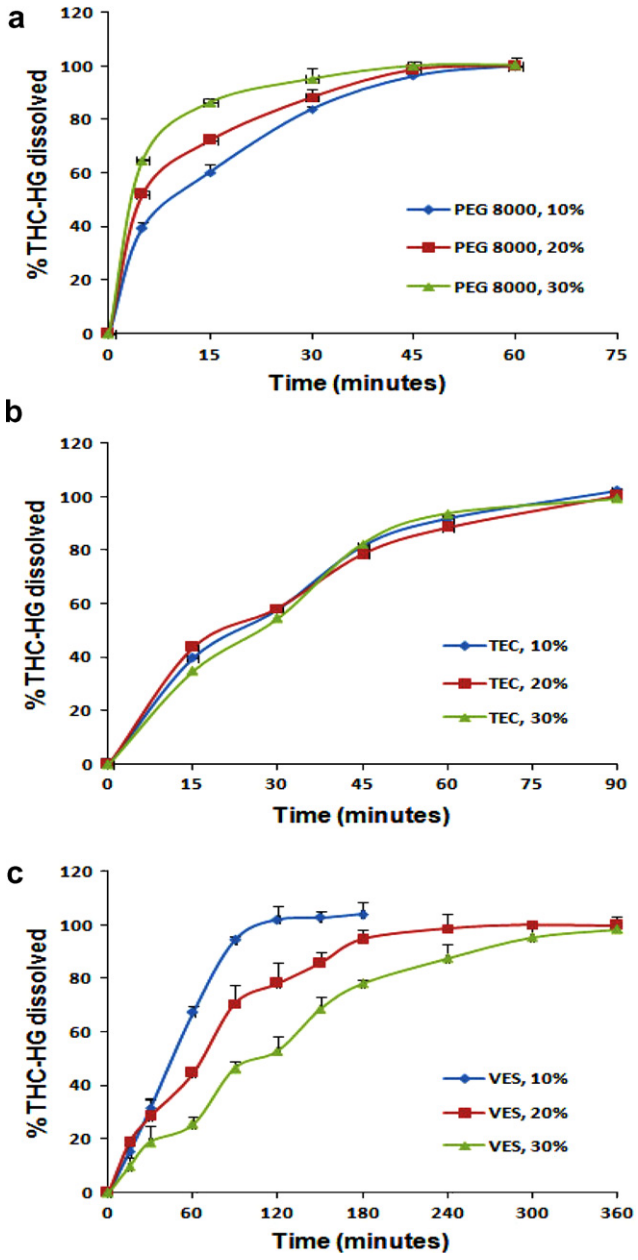


Fig. 9. Influence of (a) PEG 8000, (b) TEC and (c) VES, concentration on the release of THC-HG from PEO polymeric matrices. The matrices ($n = 3$) were fabricated at 110 °C for 7 min.

water-insoluble plasticizers increased, the resistance offered to the aqueous dissolution media penetrating into the polymeric matrix is increased, thereby slowing the dissolution rate of the prodrug.

4. Conclusions

The results of this study indicate that processing variables such as processing temperature and heating duration can have a significant influence on the stability of heat-labile drugs in polymeric matrices fabricated by hot-melt methods. All the plasticizers investigated allowed the hot-melt processing to be performed at a lower temperature, and reduced the degradation of a heat-labile ester prodrug during processing. THC-HG was most stable in PEO-VES matrices, while it exhibited maximum degradation in PEO-PEG 8000 and PEO matrices indicating that the prodrug is unstable in a hydrophilic environment. An increase in degradation of THC-HG with increasing concentration of PEG 8000 further corroborated this observation. Incorporation of citric acid increased the stability of the drug by adjusting the microenvironmental pH of the matrix close to the THC-HG's pH of maximum stability. Thus, microenvironmental pH modulation by the use of appropriate pH modifiers can provide an invaluable approach for stabilizing ester prodrugs in polymeric matrices, including those intended for oral transmucosal delivery of THC. The release of the prodrug from PEO matrices was influenced by the type and level of plasticizer system investigated. These findings emphasize the importance of selecting an appropriate plasticizer system in order to obtain a desired release profile for the intended therapeutic applications of this important medication, THC.

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