Evaluation of the application of polyethylene glycol 8000 as a plasticizer for the development of solid dispersions based on ellagic acid and Eudragit® EPO using hot melt extrusion

Evaluation de l'utilisation du polyéthylène glycol 8000 comme plastifiant pour le développement de dispersions solides à base d'acide ellagique et d'Eudragit® EPO par extrusion à chaud

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Reçu le 7 janvier 2024, accepté le 25 mars 2024 et publié le 20 juillet 2024

Abstract

Solid dispersion formation by hot melt extrusion (HME) is a widely used formulation strategy to improve the solubility and bioavailability of poorly water-soluble drugs. Despite this, they are limited by various factors such as drug-excipient miscibility, poor stability, limited drug loading and extrudability of physical drug-excipient mixtures. In this work, polyethylene glycol 8000 (PEG 8000) was used as a plasticizer for the manufacture of ellagic acid solid dispersions (EASD) with high drug loading. Indeed, ellagic acid (EA) is a polyphenolic active compound with antimalarial and other promising therapeutic activities. However, its low solubility and low permeability limit its therapeutic use. Solid dispersions formation may overcome this challenge, but its high melting point negatively influences the extrudability of its binary physical mixtures with a high drug loading rate, hence the need to use a plasticizer. Thus, five formulations consisting of EA, Eudragit® EPO and PEG 8000 in the ratio of 15:75:10 (F1), 20:70:10 (F2), 25:65:10 (F3), 15:80:5 (F4) and 20:85:5 (F5) % w/w, respectively, have been extruded, four of which were successful. The extrudates were evaluated by X-ray powder diffraction**,** FTIR spectroscopy and *in vitro* dissolution tests. Based on the results of these tests, the F5 formulation was identified as the most promising. Indeed, after 15 min of dissolution test, the dissolution rate of ellagic acid from the formulations was 62.67±3.10%, 58.74±7.23 %, 88.75±3.02% and 83.47±4.40% respectively for formulation F1, F2, F4 and F5. Moreover, the results of the FTIR spectroscopy analyses showed stronger interactions between the different constituents in the F4 and F5 formulations compared to the F1 and F2 formulations. Extruded materials of the F5 formulation, characterized by solid state nuclear magnetic resonance (ssNMR) spectroscopy and subjected to stability studies, showed good physical stability for twelve months under real-time stability study conditions and for six months under accelerated conditions.

Keywords: Ellagic acid, PEG 8000, ternary solid dispersions, hot melt extrusion, plasticizer, and dissolution.

Abbreviations:
API: Active Pharmaceutical Ingredient, ASD: Amorphous Solid Dispersions, EA: Ellagic Acid, HME: Hot Melt Extrusion, PEG 8000: Polyethylene glycol 8000, SD: Solid Dispersions, T_{deg}: degradation temperature, T_g: glass transition temperature

Résumé

La formation de dispersions solides par extrusion à chaud (HME) est une stratégie de formulation largement utilisée pour améliorer la solubilité et la biodisponibilité des médicaments peu solubles dans l'eau. Toutefois, cette stratégie de formulation est limitée par divers facteurs tels que la miscibilité médicament-excipient, la faible stabilité des dispersions solides, leur charge limitée en substance active et l'extrudabilité des mélanges physiques substance active-excipient. Dans ce travail, le polyéthylène glycol 8000 (PEG 8000) a été utilisé comme plastifiant pour la fabrication de dispersions solides d'acide ellagique (EASD) à forte charge médicamenteuse. En effet, l'acide ellagique (EA) est un composé actif polyphénolique qui possède des propriétés antipaludiques et d'autres activités thérapeutiques prometteuses. Cependant, sa faible solubilité et sa faible perméabilité limitent son utilisation

thérapeutique. La formation de dispersions solides pourrait permettre de surmonter ce problème. Toutefois, son point de fusion élevé influence négativement l'extrudabilité de ses mélanges physiques binaires à un taux de charge élevé, d'où la nécessité d'utiliser un plastifiant. Ainsi, cinq formulations composées d'EA, d'Eudragit® EPO et de PEG 8000 dans le rapport 15 :75 :10 (F1), 20:70:10 (F2), 25:65:10 (F3), 15:80:5 (F4) et 20:85:5 (F5) % w/w, respectivement, ont été extrudées dont quatre avec succès. Les extrudats ont été évalués par diffraction des rayons X sur poudre, par spectroscopie FTIR et par tests de dissolution in vitro. Sur la base des résultats de ces tests, la formulation F5 a été identifiée comme étant la plus prometteuse. En effet, après 15 min de test de dissolution, le taux de dissolution de l'AE des formulations était de 62,67±3,10%, 58,74±7,23 %, 88,75±3,02% et 83,47±4,40% respectivement pour les formulations F1, F2, F4 et F5. De plus, les résultats des analyses par spectroscopiques FTIR ont montré des interactions plus fortes entre les différents constituants dans les formulations F4 et F5 par rapport aux formulations F1 et F2. Les extrudats de la formulation F5, caractérisés par spectroscopie de résonance magnétique nucléaire à l'état solide et soumis à des études de stabilité, ont montré une bonne stabilité physique pendant douze mois dans des conditions d'étude de stabilité en temps réel et pendant six mois dans des conditions d'étude de stabilité accélérée.

Mots clés : acide ellagique, polyéthylène glycol 8000, dispersions solides ternaires, extrusion à chaud, plastifiant et dissolution

1. Introduction

Ellagic acid (EA) is a natural polyphenolic compound discovered by chemist Henri Braconnot in 1831. EA is widely distributed in many tropical and mediterranean plant species such as *Adenium obesum* (Apocynaceae), *Terminalia chebula* (Combretaceae), *Rosa rugosa* (Rosaceae) and *Punica granatum* (Lythraceae). In plants, EA exists in free form, in glycosylated and/or acylated form, or linked as ellagitannins esterified with glucose (1). It has been found that EA has important beneficial health effects against many oxidation-linked chronic diseases. Various studies have confirmed that EA has antimalarial, anti-nociceptive, anti-proliferative, anti-mutagenic, hepatoprotective, anti-diabetic, cardioprotective, promoting blood coagulation (activation of factor XII), antimicrobial or antiviral activities (2). This multifaceted health activity of EA makes it widely used in the food and cosmetic industries (3). However, its therapeutic utilization, as for example, in the treatment of malaria by oral route, remains a great challenge. Indeed, data on solubility, oral absorption, and permeability are sufficiently exhaustive to classify EA into Class IV of the Biopharmaceutics Classification System (4). Thus, EA has a low oral bioavailability, especially in humans, since less than 1% of the orally administered dose is absorbed from the gastrointestinal tract (5). This low bioavailability is mainly due to its extremely low solubility resulting from its high degree of crystallinity, with an estimated melting temperature of more than 360°C. Being soluble neither in water nor in oils, EA belongs to the group of molecules called "brick dust".

To develop its therapeutic potential, it is necessary to develop delivery systems that enhance EA solubility, dissolution rate and bioavailability. Different approaches such as complexation with cyclodextrins (6), particle size reduction (7), lipid formulations (8), and solid dispersions (SD) formation (9) have been proposed for this purpose, without leading to the therapeutic use of EA. Out of all these techniques to improve solubility, SD formulation remains one of the widely used technique due to its simplicity and ease of commercialization (10). Several methods based on different mechanisms are currently employed to generate SD, each of which has its advantages as well as limitations (11).The choice of method is selected based on the physicochemical properties of drug, nature of excipients and the intended dosage form. They can be broadly classified into solvent-based methods and melting or fusion methods. Solvent-based methods include spray drying, electrospraying, and rotary evaporation, wherein the drug and polymer are dissolved in a solvent which is then evaporated to form SD. These methods are suitable for thermolabile drugs soluble in volatile solvents. The selection of the solvent system that can solubilize the drug-polymer mixture and be compatible with them is the most difficult aspect of their utilization. Indeed, poor or partial solubility of the constituents may lead to longer processing times and non-homogenous SD (12,13). Although the fusion methods do not require the use of organic solvents, degradation of the drug is an issue due to the high processing temperature. These include microwave heating, melt agglomeration, 3D printing and HME. This latter has emerged as a pioneering manufacturing technology for pharmaceutical industries, and its utilization is overgrowing due to various advantages like favorable good powder properties, no organic solvents in the processing, small footprint of the equipment, ease of increasing batch size, feasibility and scalability from, pilot to industrial setting, and suitability of batch processing (14). Extruders mainly include single-screw extruders, twin-screw extruders and multi-screw extruders. Among them, twin screw extruders, especially corotative twin screw extruders, are widely used in the research and pharmaceutical industries owing to their superior efficiency of mixing and self-wiping action ensuring first-in-first-out material flow. In addition to the rotating screws, these equipments consist of a motor, which acts as a drive unit, an extrusion cylinder and an extrusion die. In the HME process, a mixture of drug and thermoplastic polymer is heated, softened and the melt extruded through a die under defined conditions (15). These conditions relate to processing parameters, such as

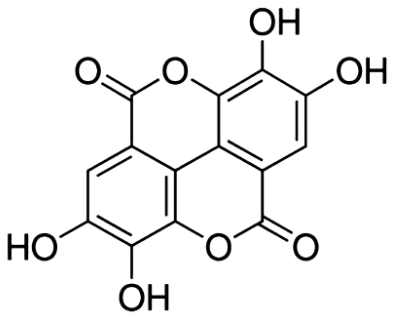
https://doi.org/10.57220/jatpb.v3i1.179

feed rate, Screw configuration, temperature, die geometry, barrel design and screw speed, which must be considered when using this method, as they contribute significantly to the quality of the final product. The major drawback of HME is the thermal exposure to the drug substance. This is most problematic with drug substances that have high melting points (above 200 °C) like EA because they may require high processing temperatures(16,17) High processing temperatures can result in degradation of the drug substance, the polymer carrier, or both. Moreover, the non-thermoplastic behavior of these drugs negatively influences the extrudability of physical mixtures, resulting in the production of SD with low drug loading rates. Indeed, extrusion is a mechanical process and the torque exerted by the screws is dependent upon the viscosity of the melt. When the torque exceeds the limit supported by the extruder, the process stops (18,19) . To overcome this limitation, some authors propose the use of a plasticizer or surfactant during extrusion to reduce the glass transition temperature of the polymer, thereby lowering the processing temperature or reducing the melt viscosity (20).
In a previous study, we conducted pre-formulation work that led to the selection of Eudragit® EPO as a suitable polymer for the development of EASD by HME (21). However, the extrudability of the physical mixtures was the main limiting factor for increasing the EA loading rate in the SD. This study was therefore carried out with the aim of increasing the loading rate of EA in SD and assessing the impact of using polyethylene glycol 8000 as a plasticizer on the extrudability of EA/ Eudragit® EPO physical blends, as well as on the pharmaceutical performance of the resulting extrudates.

2. Materials and methods

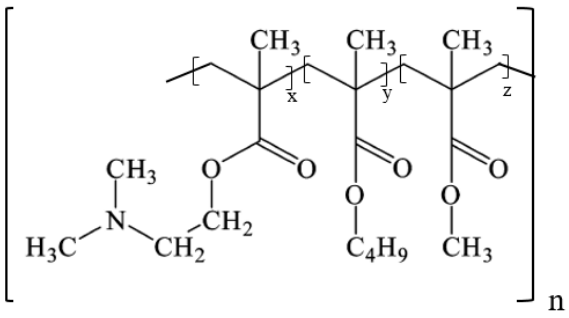
2.1 Materials

Ellagic acid dihydrate (98%) was purchased from Fluorochem Ltd Unit 14, (Graphite Way Hadfield, Derbyshire, United Kingdom). Eudragit® EPO (Dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate), soluble in water at pH < 5, was a kind gift from Evonik Corp (Darm-stadt, Germany). Poly (ethylene glycol) (PEG 8000) were obtained from Sigma Aldrich and ultrapure water was produced by a Milli-Q system (Millipore, Bredford, MA, USA). Acetonitrile was HPLC grade and purchased from J.T. Baker (Gliwice, Poland). Hydrochloric acid (37% wt. %) for analysis and monosodium phosphate (Ph. Eur, Merck, Darmstadt, Germany) were purchased. All the other reagents and solvents were of analytical grade. The names, structures, and physicochemical properties of the polymers and drug used are given in **Figure 1**



Ellagic acid (3,7,8-tetrahydroxy-chromeno [5,4,3-cde]-chromene-5,10-dione

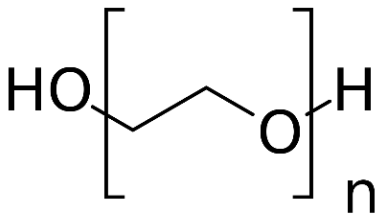
Molar weight: 302.197 g/mol (43)



Eudragit® EPO (dimethylaminoethyl methacrylate–butylmethacrylate–methylmethacrylate copolymer (50/25/25)**)**

T_g ≈ **55**°C Molar weight ≈ 47000 g/mol

T_{deg} ≈ 250°C (42)



Polyethylene glycol

Tm≈ 60°C**,** Molar weight ≈ 8000 g/mol

T_g ≈ -100°C (Li et al., 2020), T_{deg} ≈ 300°C

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Figure 1: Names, structures, and physicochemical properties of polymers and ellagic acid

2.2 Methods

2.2.1 Preparation of solid dispersions

Ternary (EA: Eudragit® EPO: PEG 8000) physical mixtures (50 g) were prepared in a mortar with pestle in the ratio of 15:75:10 (F1), 20:70:10 (F2), 25:65:10 (F3), 15:80:5 (F4) and 20:85:5 (F5) % w/w, respectively. The ternary physical mixtures were then extruded using an 18 mm twin screw hot melt extruder (L/D=24, Scamex1, Crosne, France) with a standard screw configuration containing 2 kneading zones and a 2 mm die. The powder was loaded into the volumetric feeder of the extruder which feeds the barrel through two screws rotating at 4 rpm. The processing screw rate was set at 100 rpm and temperatures of the five-heated barrel and die zones were set as follows: zone 1= 160°C, 2= 165°C, 3= 170°C, 4= 175°C and 5=180°C. After cooling to ambient conditions, the extrudates were milled using a commercial grinder (Frewitt FreDrive-Lab HammerWitt-Lab, Granges-Paccot, Switzerland) and then passed through a sieve with mesh openings of 0.35 um. The powder was finally collected for characterization.

2.2.2 Characterization of milled extrudates

The milled extrudates were characterized by X-ray diffraction, fourier transform infrared spectroscopy and dissolution testing under non-sink conditions. In addition, the drug content in each extruded formulation was determined. All these studies were carried out using the method reported by Nyamba et al. (21). The most promising formulation resulting from these tests was characterized by solid state nuclear magnetic resonance spectroscopy and subjected to stability tests using the method described below.

2.2.3 Solid state nuclear magnetic resonance (ssNMR) spectroscopy

Carbon-13 ssNMR spectra were acquired on a Bruker Avance III HD NMR spectrometer operating at B0 = 9.4 T, with corresponding 1H and 13C resonance frequencies of ν0 (1H) = 400.1 MHz and ν0(13C) = 100.6 MHz. Samples of EA, Eudragit® EPO, PEG 8000 and extrudates were packed in 4 mm o.d. zirconia rotors with Kel-F caps under ambient atmosphere, and experimental 13C NMR spectra were acquired at natural abundance using a 4 mm double channel (X/H) Bruker MAS probe. A contact time of 4 ms was optimized using a crystalline sample of EA and was used for the acquisition of all 13C CP/MAS NMR spectra. Tetramethylsilane was used as shift reference.

2.2.7 Stability Studies

Stability studies were carried out on milled extrudates packed in an airtight container and stored in stability chambers (MEMMERT Pharma 600 ICH 260, Germany) at 40°C ±2°C / 75% ± 5% RH (accelerated) for 6 months (6M) and at 30°C ±2°C / 65% ± 5% RH (Real-Time) for 12 months storage conditions following the ICH Q1A guidelines. The content and dissolution were determined by HPLC as described by Nyamba et al. (21) at M1, M3 M6 and M12 and compared with the original state (M0).

2.2.8 Statistical analysis

The data statistics were performed using Graphpad Prism version 5.03 software (GraphPad Software, Inc., La Jolla, CA, USA) by two-way ANOVA test and Tuckey tests. The results are presented as mean ± SD. Statistical differences were considered significant between the groups if the p-value was < 0.05. All experiments were carried out in triplicate (n=3).

3. Results and discussion

3.1 Plasticizer utilization

Plasticizers are typically small molecules that act to increase the free volume between polymer chains, resulting in a depression of the Tg and the melt viscosity (22). The ideal plasticizer should be highly compatible with the polymer, stable under the hot-melt extrusion conditions and sufficiently lubricating and stable when present in the final product. In addition, even more important for pharmaceutical applications, they should comply with environmental, health and safety regulations. Although a lot of plasticizers exist for chemical applications, only limited choices of approved plasticizers for pharmaceutical industry are therefore available (23) . A review of the literature based on these criteria led to the choice of polyethylene glycol 8000 **(Figure 1)** as the plasticizer in this study. Indeed, polyethylene glycol 8000 is a water soluble, biodegradable, and biocompatible polymer, solid at room temperature with a solubility parameter (21.7 $MPa^{1/2}$) close to that of Eudragit® EPO (20.15 $MPa^{1/2}$). Generally, plasticizer polymer systems with similar solubility parameter values are predicted to be more miscible. Plasticizer-polymer mixtures with the solubility parameter difference, $\Delta\delta < 7.0$ $MPa^{1/2}$ are found to be miscible whereas systems with $\Delta\delta > 10.0$ $MPa^{1/2}$ are likely to be immiscible (24). Estimation of plasticizer-polymer miscibility based on the difference in the solubility parameter values is still one of the most applied approaches in

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the academia and pharmaceutical industry owing to its relative simplicity (14,20). Furthermore, Desai et al. 2018 showed that PEG 8000 at 10% w/w resulted in a halving of the mechanical torque when producing Eudragit® EPO-based ternary SD (Desai et al., 2018). The reduction of the mechanical torque (% motor load) is a good indicator to measure the plasticization efficiency. Finally, FTIR analysis of Eudragit® EPO /PEG 8000 physical mixtures in proportions of 95/5% w/w and 90/10% w/w showed good interaction between the two polymers (**Figure 3**).

3.2 Preparation of solid dispersions

Four of the five ternary physical mixtures were successfully extruded under the employed extrusion process parameters. These were formulations F1, F2, F4 and F5. These formulations were extruded with a motor torque of 19 Nm, 34 Nm, 28 Nm and 37 Nm respectively. In a previous study, we showed that the motor torque during the extrusion process, exceeded 40Nm (the extruder limit) when the proportion of EA in binary mixtures EA/Eudragit® EPO exceeded 12% w/w, indicating that processing was not feasible (21). The presence of PEG 8000 as plasticizer therefore enabled the extrusion of formulations F1, and F2; F4 and F5 with maximum torque reductions of 52.5% and 15%, 30% and 7.5% respectively. However, the F3 formulation could not be extruded. The non-extrudability of this formulation was related to the amount of EA contained in the ternary mixture, which produced a torque > 40Nm during processing despite the presence of the plasticizer. Increasing the proportion of plasticizer, therefore, improves the extrudability of ternary mixtures. However, adding a high proportion of plasticizer that remains in the product, can increase the total weight of the formulation and result in large and unacceptable dosage forms (25). Moreover, the properties and performance (physicochemical stability, dissolution, T_g, hygroscopicity or appearance) of the product may be affected (26). Ideally, a plasticizer should be capable of providing the desired plasticizing effect and then removed from the formulation to mitigate any negative effects before final processing. For this reason, some authors advocate the use of supercritical carbon dioxide (scCO2), low-boiling solvents or reagents that can evaporate or sublimate as plasticizers during the extrusion process (27). However, the use of these plasticizers may require special handling and equipment modifications or leave toxic residues in the final product. Therefore, during preclinical development, the choice and optimal loading of the plasticizer in the formulation must be studied on a case-by-case basis (20).

3.3 Drug content

The EA content in the milled extrudates of the four formulations was evaluated by HPLC. The values obtained were respectively 96.5% ±1.23, 93.37% ±1.54, 94.43%± 1.15 and 92.65%±2.16 for the formulations F1, F2, F4 and F5, in relation to the theoretical content. Although there was no statistically significant difference between these values ($p< 0.05$), it is well known that increasing motor torque can lead to a reduction in drug content due to lower drug degradation caused by higher stress. Furthermore, the drug contents of ternary extrudates are slightly higher than those obtained previously with binary extrudates (89.86 ±1.15% to 90.60 ±1.27%) based on EA and Eudragit® EPO produced with the same extrusion parameters (21). This could be explained by the better processability of the ternary mixtures by HME.

3.4 X-ray powder diffraction (XRPD)

The diffractograms of the raw materials and successfully the extruded formulations are in **Figure 2.** X-ray powder diffraction is a convenient technique for analyzing the crystal structures of organic, inorganic and polymeric materials (30). It has been described as the gold standard in characterizing pharmaceutical materials in the solid state (31). Indeed, every crystal has its unique arrangement of atoms and repeating units. When X-rays are applied, these atoms are irradiated and generate a series of distinct peaks, which is used to unambiguously identify the crystalline components (32). Thus, X-ray powder diffraction was used to confirm or deny the crystalline nature of the obtained EA ternary SD. EA showed peaks at 2 theta of 20.90°, 25.14°, 26.43°, 29.29° and the most intense peak was observed at 28.30°. A similar result was reported by Li et al., (32) indicating the crystalline nature of the pure EA. As a semi-crystalline polymer, the diffractogram of polyethylene glycol, showed a peak at 2 theta of 19.20° and 23.30°. These peaks were absent on the diffractograms of the different formulations except for the F2 formulation. On the other hand, we noted the presence of numerous characteristic EA peaks in these diffractograms highlighting the presence of residual drug crystals in all formulations. The presence of these crystals proves the inability of the process parameters and polymer blend to amorphized all the EA with an extrusion temperature of 180 °C. However, the intensity and width of the peaks differed among the formulations, indicating different degrees of crystallinity of the extrudates.

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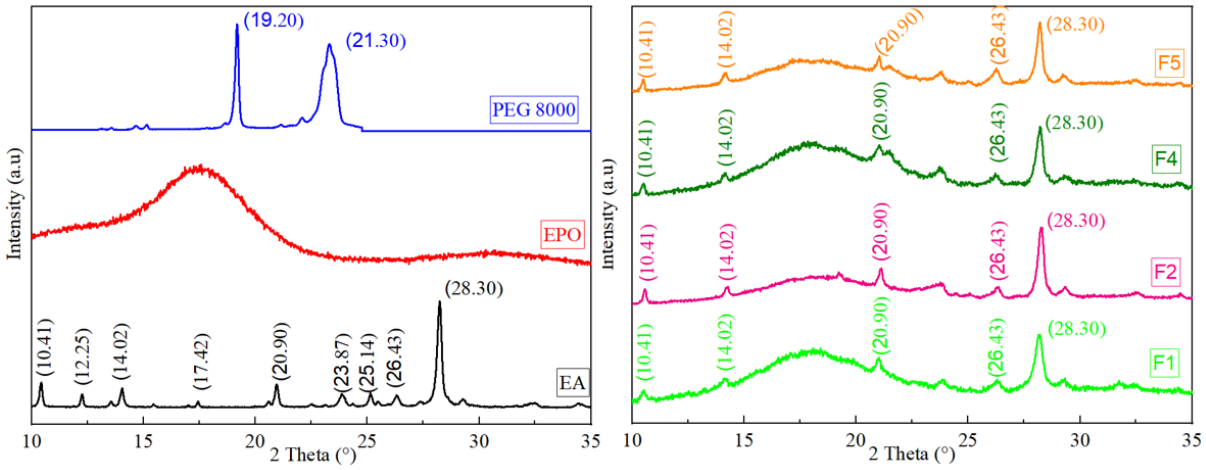

Figure 2 : Diffractograms of the raw and extruded materials. EA: Ellagic acid, EPO: Eudragit® EPO, PEG 8000: Polyethylene glycol 8000 and EA/EPO/PEG 8000 in the ratio of 15/75/10 (F1), 20/70/10 (F2), 15/80/5 (F4) and 20/75/5 (F5) % w/w.

3.5 Fourier transforms infrared spectroscopy (FTIR)

FTIR was used to study the possible interactions of Eudragit® EPO with polyethylene glycol 8000 and to study the interactions between EA, Eudragit® EPO and polyethylene glycol 8000 in the successfully extruded formulations. The results are shown in **Figure 3 (A et B)**. The structures of the carrier, drug and plasticizer are shown in **Figure 1**. PEG 8000 showed a characteristic broad spectrum of O-H stretching vibration from 3300 to 3600 cm^{-1}, C-H stretching of OC_2H_5 groups from 2800 to 2900 cm^{-1}, and C-O stretching from 1000 to 1200 cm^{-1}. All these bands disappeared in the spectra of the physical polymer/plasticizer mixtures at 5 and 10 % w/w thus showing the involvement of these groups in the interaction with the polymer. The FT-IR spectrum of Eudragit® EPO showed a strong band at 1722 cm^{-1}, corresponding to the valence vibrations of the carboxyl groups of the three monomers. The bands at 2951 and 2875 cm^{-1} are attributed to asymmetric and symmetric CH_3 groups, those at 2822 and 2767 cm^{-1} to the dimethyl amino groups and the one at 1447 cm^{-1} to the C-H bending vibration for the methyl group. The bands at 1270 and 1238 cm^{-1} are due to the C-O stretching vibration of the ester group and the one at 1153 cm^{-1} is attributed to the C-N stretching absorption of the aliphatic amines and/or the C-O stretching vibration of the ester groups. The band at 1722 cm^{-1}, decreased in intensity in the spectra of the physical polymer/plasticizer blends and those at 1270 and 1238 cm^{-1} changed in wavenumber and appear at 1265 and 1240 cm^{-1}. These changes show the involvement of the carboxyl and C-O groups in the polymer/plasticizer interaction. The spectra of Eudragit® EPO, PEG 8000 and their physical mixture are shown in **figure 3 A**.

Pure EA showed six characteristic bands between 4000 and 1500 cm-^{1}. The first two bands at 3474 cm^{-1} and 3151 cm^{-1} and the third one at 1717 cm^{-1} correspond to the valence vibrations of the hydroxyl (O-H) and ketone (C=O) groups, respectively. The last three bands at 1615, 1582 and 1507 cm^{-1} correspond to the C=C bond of the aromatic ring. In the fingerprint region between 1500 and 650 cm^{-1}, several bands were also present. The bands at 1448 and 1398 cm^{-1} correspond to the C-H bond deformation vibrations and those at 1324 and 1033 cm^{-1} are the symmetric and asymmetric vibrations of the C-O bond. The band at 1189, 1109 and 753 cm^{-1} are respectively the vibration of the phenolic C-O bond, the C-C bond, and a substituted aromatic ring at position 4. These results are similar to those of Savic et al (33).

In the FTIR spectra of the F1 and F2 formulations, the bands of the O-H group of EA at 3474 and 3135 cm-1 were present, suggesting a weak involvement of this group in the interactions between the different constituents. In the spectra of the formulations F4 and F5 on the other hand, the first band appeared with a very weak intensity while the second band was absent suggesting a stronger involvement of the OH group in the interactions. In a previous study, we demonstrated that EA and Eudragit® EPO interacted by the formation of H-bonds between the carboxyl groups of Eudragit® EPO which accept the H atoms of the OH groups of EA. Other differences related to the appearance of the characteristic bands of EA remain between the spectra of the four formulations. These were notably the bands at 1615, 1582 and 1507 cm-1 corresponding to the C=C bond of the aromatic ring which appeared with a stronger intensity in the F1 and F2 formulations than in the F4 and 5 formulations. The presence of PEG 8000 in the four formulations, was materialized by the appearance around 2878 cm^{-1} of its C-H stretching vibration band of OC_2H_5 group with a variable intensity. All these differences observed between the spectra show globally a weaker inter-component interaction in the F1 and F2 formulations than in the F4 and 5 formulations, which could explain the heterogeneous pharmaceutical performances obtained during the dissolution tests. Indeed, the rate and speed of dissolution of drugs from solid dispersions are largely dependent on polymer-drug interactions (34). The relatively modest pharmaceutical performances of formulations F1 and F2 (containing 10%

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PEG 8000) compared to formulations F4 and 5 (containing 5% PEG 8000), could be explained by a change of the difference in δ values (Δδ) between the polymer-plasticizer mixture and EA, thus reduce the interactions between the drug and this mixture. Indeed, a similar finding was made by Jia Liu et al. who showed that combinations of Eudragit® EPO with Kollidon® VA64 or Soluplus® appear to increase the Δδs between carbamazepine and polymer blends, which does not imply improved miscibility (35).

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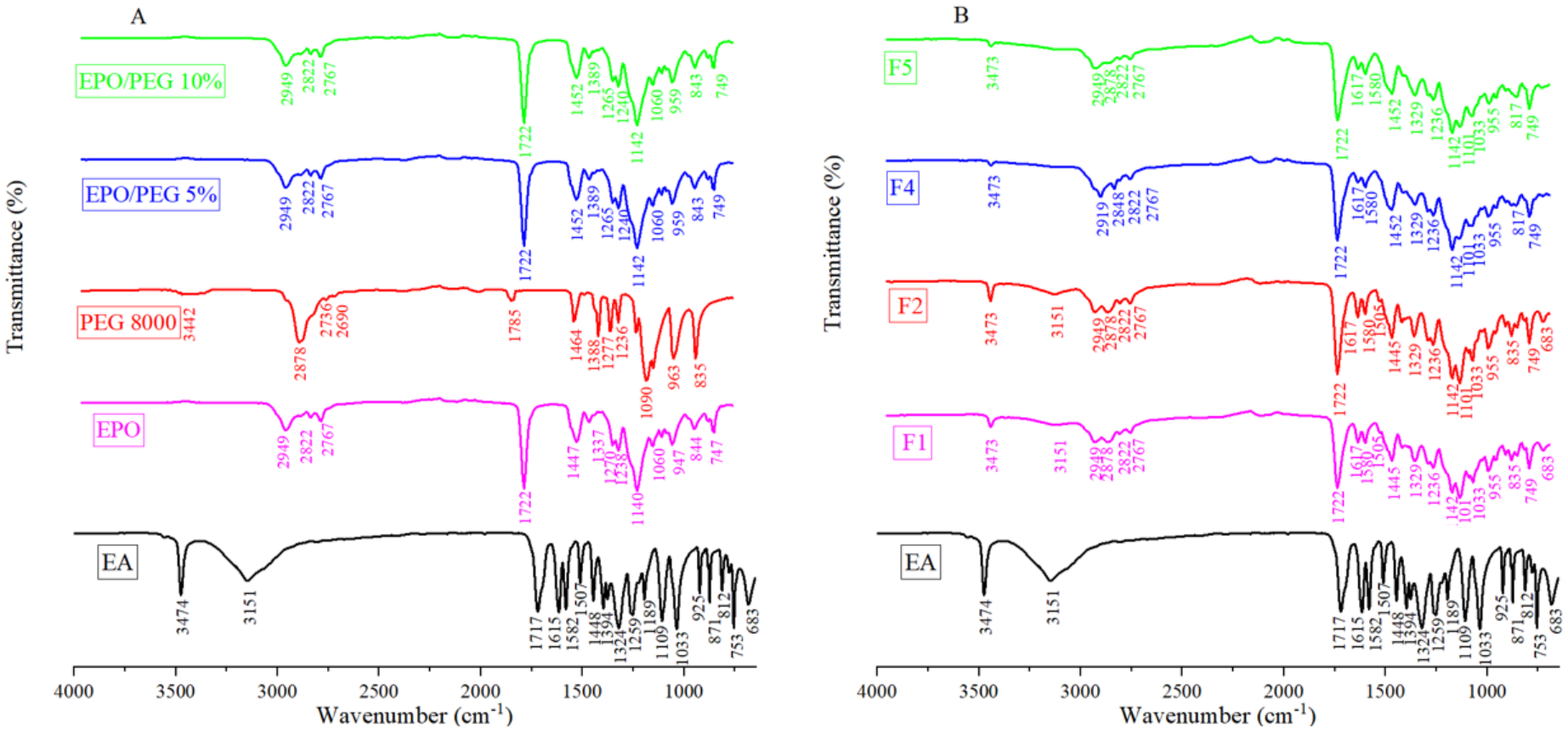

Figure 3 : The FT-IR spectra: (**A**) Ellagic acid (EA), Eudragit® EPO (EPO), Polyethylene glycol 8000 (PEG 8000), physical mixture Eudragit® EPO/ Polyethylene glycol 8000 at 5% w/w (EPO/PEG 5%) and at 10 % w/w (EPO/PEG 10 %); (**B**) Ellagic acid (EA), EA/EPO/PEG 8000 in the ratio of 15/75/10 (F1), 20/70/10 (F2), 15/80/5 (F4) and 20/75/5 (F5) % w/w.

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3.6 In vitro non sink dissolution testing

This study was conducted to evaluate the influence of both PEG 8000 and the drug loading on the pharmaceutical performance of the successfully extruded formulations (F1, F2, F4 and F5). The dissolution profiles of EA from these formulations in HCl 0.1N media are presented in **Figure 4**. The EA release was much faster from all the formulations than that from the pure EA powder. Furthermore, formulations containing 5% w/w PEG 8000 showed a higher dissolution rate of EA than those containing 10% w/w PEG 8000. Indeed, the dissolution rates of EA obtained from the F4 and F5 formulations at 15 min were 88.75±3.02% and 83.47±4.40% respectively before reaching maximums of 90.08± 3.17 and 86.50±5.54% at 60 min. For the formulations containing 10% w/w PEG 8000 (F1 and F2), the dissolution rates were 62.67±3.10 and 58.74±7.23 % respectively at 15 min before reaching maximums of 72.73 ± 4.0 % and 62.28 ± 3.78 % at 30 min. In addition, there was a tendency for recrystallization of dissolved EA from these formulations. The proportion of 10% of plasticizer had therefore a negative effect on both the release rate of EA and on the stability of its supersaturated solution. The 15% drug loaded formulations (F1, F4) showed a slightly higher dissolution rate than the 20 % drug loaded formulations. However, no statistically significant difference ($p < 0.05$) was observed between the dissolution rates of the F4 and F5 formulations unlike the F1 and F2 formulations. In contrast to the binary SD developed in our previous study, the dissolution of EA from all formulations was less rapid and incomplete. This could be explained both by the increase of the EA loading rate in the formulations and by the presence of the plasticizer. Indeed, one of the drawbacks of the production of formulations with high loading rate by the hot extrusion method is the possible presence of residual drug crystals with low solubility. This residual crystallinity, therefore, limit the dissolution rate of the drug from the formulations. The presence of such residues in our formulations has been confirmed by X-ray diffraction analysis (**Figure 2**). In general, low drug loading (or high polymer-drug ratio) favors congruent release of the drug with polymer, thereby facilitating the drug dissolution (36). On the other hand, low drug loading formulations lead to large dosage forms for all but the more potent compounds, which is undesirable from a patient compliance perspective and can provide a marketing disadvantage. Therefore, a balance must be struck between a drug loading rate high enough to achieve desirable dosage forms from the point of view of patient compliance and a drug loading rate low enough to achieve good drug release during dispersion development (37). Regarding the presence of PEG 8000 as a plasticizer in the formulations, it is well known that plasticizers incorporated into the pharmaceutical polymers not only facilitate the thermal processing of polymers but also modify the mechanical properties and water absorption behavior of the polymers (29). All these properties affect the strength and integrity of the polymer, 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 polymer. This difference could be a result of solubility and affinity of the plasticizer to the polymer (38). When there is a strong interaction between the polymer and plasticizer, this can reduce the polymer-drug interaction and therefore decrease the ability of ternary SD to form and maintain supersaturated drug solutions, as was the case in this study. Indeed, Chen et al.; 2016, studied the impact of polymer type and drug-polymer interaction on the dissolution rate of SD, and concluded that the drug release rate is correlated with polymer release rate and the strength of drug-polymer interaction in SD (39). With similar solubility parameters, Eudragit® EPO/PEG 8000 blends show strong interactions. This was demonstrated by FTIR spectroscopy characterization of Eudragit® EPO/PEG 8000 physical mixtures at 5 and 10% w/w **(Figure 3)** Based on its pharmaceutical performance, loading rate in EA and PEG 8000, the F5 formulation was selected as the most promising.

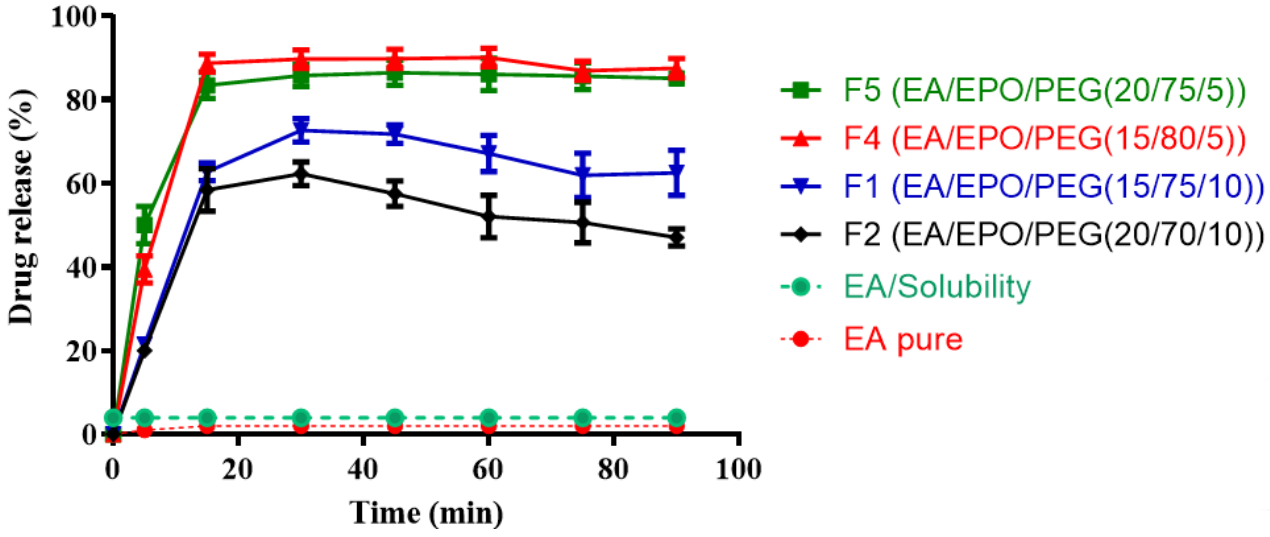

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Figure 4 : Dissolution profiles of successfully the extruded formulations to study the influence of both PEG 8000 and the drug loading on the release of EA. EA: Ellagic acid, EPO: Eudragit® EPO, PEG 8000: Polyethylene glycol 8000.

3.7 Solid state nuclear magnetic resonance (ssNMR) spectroscopy

The ssNMR results performed with the extruded materials of the F5 formulation are shown in the **Figure 5**. ssNMR is non-destructive technique which provides qualitative and quantitative information about solid dispersions. The 13C ssNMR spectrum of crystalline EA is consistent with that previously published in the literature (2). Peak broadening was observed in the SD spectrum, indicating that the components had reduced crystallinity. The NMR spectrum of crystalline EA shows sharp narrow peaks, which became particularly broad in the 13C NMR spectrum of SD. The differences between the extrudate and crystalline EA spectra are particularly noticeable around 120 ppm (one peak for EA in SD; two peaks for crystalline EA) and146 ppm (one broad peak for EA in SD; three peaks for crystalline EA). Several peaks from the PEG 8000 and Eudragit EPO excipients overlapped with those from the ASD spectra. The broad peaks with chemical shift(s) almost matching those of crystalline EA in the 13C NMR spectrum of ASD confirm that EA had reduced crystallinity in the extrudates. These results are consistent with the powder X-ray diffraction studies.

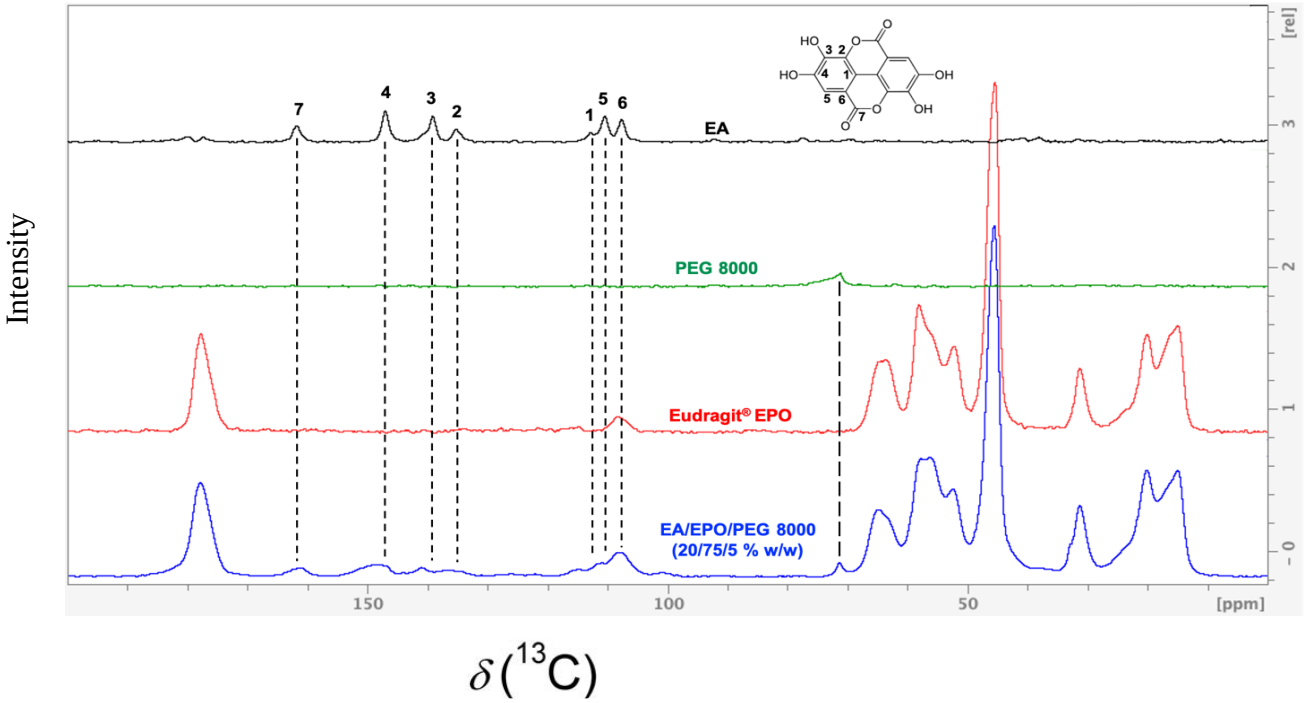

Figure 5 : 13C spectra of the raw and extruded materials: EA/EPO/PEG 8000 (20/75/5 % w/w) ternary SD spectra (blue), crystalline ellagic acid (green), Eudragit® EPO (red), Polyethylene glycol 8000 (black).

3.8 Stability Studies

Stability tests are routine procedures that are conducted under different conditions for investigating the effect of variation in temperature, humidity, light intensity, and time on pharmaceutical products (40). They allow the definition of storage conditions under which products maintain their physical, chemical and toxicological characteristics during their shelf life. Stability studies are therefore crucial in the development of pharmaceutical products to ensure the quality, safety and efficacy of the pharmaceutical product as expected by patients (41). In this study, stability tests were performed on milled extrudates of the most promising formulation (F5 composed of EA/EPO/PEG 8000 in the ratio 20/75/5 % w/w) in order to evaluate over twelve months, the physical and chemical stability of this plasticized ternary SD at 30°C ±2°C / 65% ± 5% RH and at 40°C ±2°C / 75% ± 5% RH for six months. Indeed, one of the concerns with the use of plasticizers as processing aids for HME is that they plasticize the resulting dispersion, which could have an impact on the physical stability of the drug substance (25,27) during storage. Therefore, the stability of plasticized ternary dispersions is of great interest for the development and the choice of optimal formulation. The dissolution profiles of EA from milled extrudates subjected to accelerated and real-time stability tests are presented in **Figure 6 A and B.** There is no significant difference ($p < 0.05$) in in-vitro non-sink dissolution profiles of EA from the stability samples compared to that of the initial sample, suggesting

that the prepared SD formulations were stable under the conditions of the stability study. The ability of milled extrudates to remain physically stable can be explained by the good polymers-drug interactions but also by their crystalline nature. The results of the evaluation of the EA content of the stability samples are presented in Figure **6 C**. These results show that the EA content was not affected by the conditions of the stability studies. Indeed, the EA recovery at each sampling was between 95 and 106%.

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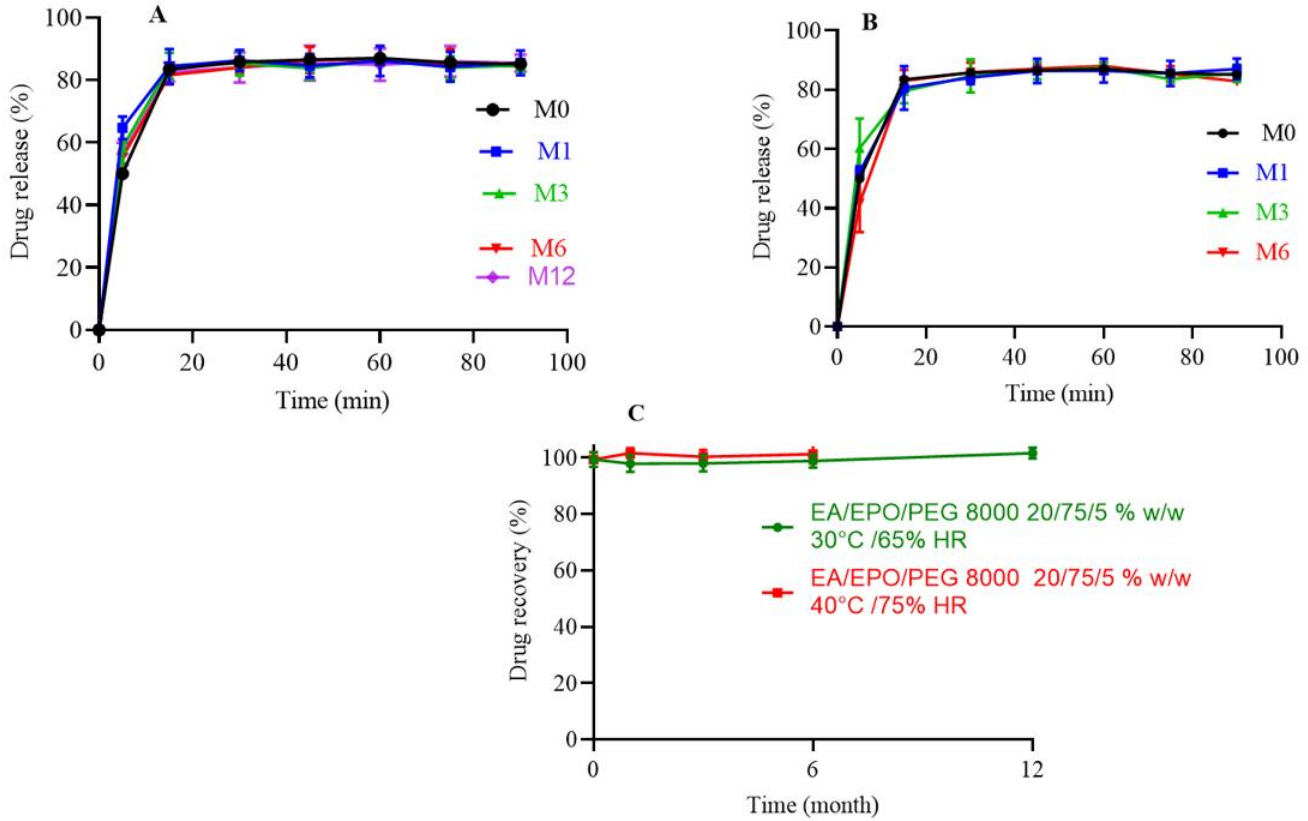

Figure 6 : Content and dissolution profiles of ellagic acid obtained from the stability extrudates : **A** dissolution profiles of ellagic acid from extrudates subjected to real-time stability study conditions (30°C ±2°C / 65% ± 5% RH) at the initial time (M0), after one month (M1), three months (M3) and six months (M6) of exposure ; **B** dissolution profiles of ellagic acid from extrudates subjected to accelerated stability study conditions (40°C ±2°C / 75% ± 5% RH) at the initial time (M0), after one month (M1), three months (M3) and six months (M6) of exposure ; **C** evolution of averages of ellagic acid contents in the extrudates subjected to real-time stability study conditions (green) and to accelerated stability study conditions (red).

https://doi.org/10.57220/jatpb.v3i1.179

4. Conclusion

In this study, the effect of polyethylene glycol 8000 used as a plasticizer was evaluated on the extrudability of ternary mixtures containing an increasing amounts of EA. In addition, the combined effects of increasing the EA loading rate and the presence of PEG 8000 at different proportions were evaluated. Thus, four formulations were successfully extruded, and the dissolution profiles of the EA obtained from the different extrudates were heterogeneous. Formulations containing 5% w/w PEG 8000 performed better than those containing 10% w/w. These results show that the proportion of plasticizer can be a factor negatively affecting the pharmaceutical performance of extrudates. Formulations F4 and F5 containing 5% PEG 8000 but 15 and 20% EA loading respectively showed no statistically significant difference in terms of EA release rate, unlike formulations F1 and F2. The X-Ray diffraction analysis has shown that the successfully extruded formulations all contained residual crystalline drug. The F5 formulation was selected as the most promising among the successfully extruded formulations based on its pharmaceutical performance achieved by dissolution test and its loading rates in EA and PEG 8000.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Authors would like to acknowledge the Academy for research and Higher Education- Development Cooperation Committee (ARES-CCD) for the financial support.

Credit authorship contribution statement

Isaïe NYAMBA: investigation, Visualization, Methodology, Writing original draft. Alexis M W NEMBOT: Writing – review & editing. Charles B SOMBIE: Writing review & editing. Hermine ZIMÉ DIAWARA: Writing review & editing. Josias B.G. YAMÉOGO: Writing review & editing. Anna LECHANTEUR: Validation, Supervision, Writing – review & editing. Christian DAMBLON: Writing review & editing. Rasmané SEMDE: Validation, Supervision, Writing – review & editing. Brigitte EVRARD: Validation, Supervision, Writing – review & editing.

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