Stability ring lactone study of camptothecin and irinotecan in artificial media and Human plasma

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Abstract

Camptothecin is a promising anti-colorectal cancer agent. However, its use in clinical medicine is compromised by its low solubility and high instability in physiological media. The present work aims to study the chemical stability of the camptothecin lactone ring (alone, encapsulated, and in solid dispersion) while comparing it to that of a reference analog irinotecan. This stability is studied in acidic (pH 2) and physiological (saline) media and Human plasma by high performance liquid chromatography (HPLC). Stability tests carried out on CPT alone, encapsulated and in solid dispersion in artificial media and Human plasma demonstrate that ternary systems [CPT/β-CDs/PEG 6000 and CPT/PM-βCDs/PEG 6000] can lead to the protection of CPT lactone ring in physiological media and Human plasma for 72 hours. This protection is essential to the camptothecin therapeutic effect when administered in pharmaceutical form.

Keywords: *Camptothecin, Chemical Stability, Colorectal Cancer, Cyclodextrin, Irinotecan, Polyethylene Glycol, Solid Dispersion.*

I. Introduction

One of the most promising anti-colorectal cancer agents is camptothecin (CPT, Figure 1), which is a pentacyclic alkaloid with a pyrroloquinoline motif [1]. This pale-yellow crystalline powder is extracted from an Asian tree called Camptotheca Acuminata. Camptothecin is a non-competitive inhibitor of topoisomerase I, as it binds neither to the enzyme itself nor to DNA but binds to both at the same time (DNA-Topoisomerase I) to form a reversible ternary complex (DNA-Topoisomerase I-Camptothecin) called cleavable complex which will inhibit the action of the enzyme by irreversibly cutting DNA in multiple locations, leading to cell death. Despite its remarkable anticancer activity, camptothecin is insoluble in water and physiological media, its dissolution is obtained in dimethyl sulfoxide (DMSO). Moreover, it is unstable in physiological media due to the hydrolysis of its lactone ring leading to the loss of its biological activity [2].

Thus, despite the proven therapeutic potential of camptothecin (CPT), it has not been exploited in therapy. However, it has been the source of a more soluble and less unstable analog such as irinotecan (CPT 11, Figure 1) [3].

However, other solutions have been studied by researchers such as encapsulation by cyclodextrins, nanofibrous silica microparticles, liposomes, and solid dispersion in polyethylene glycol (PEG) [4-7]. In addition, numerous research has been carried out to overcome the lack of solubility and stability of camptothecin in physiological media, using encapsulation with cyclodextrins (CDs), solid dispersion in polyethylene glycol (PEG), and/or the formation of ternary systems (CPT-CD-PEG) [4].

Cyclodextrins (CDs) are cyclic oligosaccharides composed of 6 to 12 glucopyranosyl subunits linked by α-(1,4) glycosidic bonds. The CDs containing more than 8 glucopyranosyl units are not stable enough for pharmaceutical use. The most common CDs are the native ones (cyclodextrins no branching other than the glycosidic ring) α, β, γ which have 6, 7, and 8 glucopyranose residues respectively, all having the chair configuration. The CD is characterized by a "crown" structure and is in the form of a truncated cone ring with primary and secondary hydroxyls on the outside and hydrogen, carbon and ether-oxide bonds on the inside, their structures are given in Figure 2 [8].

To improve the properties of cyclodextrins, numerous derivatives have been developed: Hydroxypropyl-beta-CD (HP- β -CD), Per methyl-beta-CD (PM- β -CD), Sulfobuthyl-beta-CD (SBE- β -CD), Hydroxypropyl-gamma-CD (HP- γ -CD). The chemical modifications concern the hydroxyl groups, which are substituted in varying numbers depending on the type of native cyclodextrin [9].

Because of the truncated cone structure and the particular position of the hydroxyls, cyclodextrins are amphiphilic and therefore have two distinct zones of polarity. The outside of the cavity and the ends are polar. Thus, it is the primary and secondary hydroxyls on the edges of the cone that make this molecule soluble in water. On the other hand, the inner of the cavity where oxygens are found is less polar. This more hydrophobic area will play an important function in the inclusion of the hydrophobic host molecules.

Indeed, FATMI et al. [4,5] have established that camptothecin encapsulated by cyclodextrins becomes more soluble especially when using methyl-beta-CD, indicating a potential increased bioavailability.

Also, it is now known that solid dispersion (SD) increases the solubility of drugs converting them into amorphous crystals, by reducing their particle size and by increasing their wettability [10]. In this process polyethylene glycols (PEGs, Figure 2) are very often used as water-soluble polymeric matrix [10], they are non-toxic and biocompatible. The Food and Drug Administration (FDA) has approved their use in intravenous, oral, and dermal pharmaceutical preparations [11].

A. Paudel et al. [12] have demonstrated that it is possible to improve the solubility of hydrophobic molecules in general and naproxen in particular through solid dispersion. X. Wang et al also used solid dispersion in PEG and Polyvinyl pyrrolidone (PVP) to improve the solubility of two highly lipid soluble molecules, namely, itraconazole and indomethacin, resulting in binary (PA/polymer) and ternary systems [(PA/surfactant)/polymer] with very satisfactory dissolution percentages [13].

The present work aims to study the chemical stability (lactone ring) that guarantees the anticancer activity of camptothecin (alone, encapsulated and in solid dispersion) while comparing it to that of a reference analog CPT 11. This stability is studied in acidic, physiological, and human plasma by high-performance liquid chromatography (HPLC).

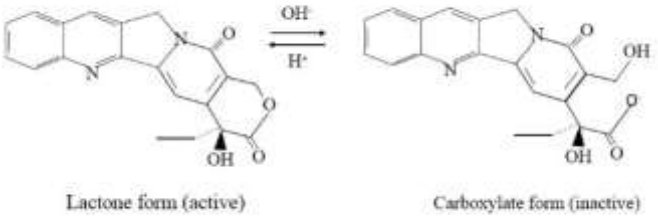

Figure 1: Camptothecin and irinotecan structure.

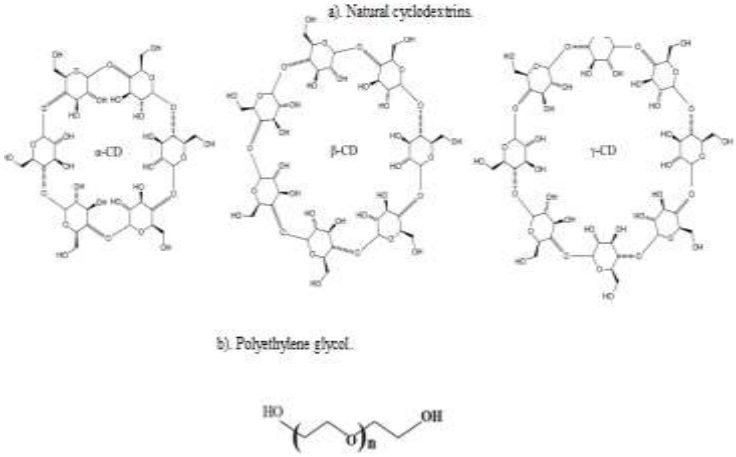

Figure 2: Chemical structures a) Natural cyclodextrins. b) Polyethylene glycol.

II. Material and methods

II.1. Materials

- Camptothecin (M_w 348.11 g/mol) and irinotecan (M_w 586.678 g/mol) were purchased from Shenzhen Boda Natural Product laboratory (P. R. China).
- Cyclodextrins: β-CD and PM β-CDs were obtained from Roquette Fréres (France).
- Polyethylene glycol 6000 was obtained from BASF (Germany).
- All reagents were of analytical grade.

II.2. Methods

Complexes and solid dispersion preparations

- Binary system (CPT/CDs inclusion complexes and CPT/PEG 6000) preparation

Cyclodextrins and CPT (1:1 molar ratio) were dissolved in 50 ml of ethanol, the mixture was left under agitation for 1 hour protected from light. After drying at 45°C for 1 hour, the powder was preserved in a desiccator [4].

- Ternary system (Solid dispersion of CPT complexes in PEG 6000) preparation

Nine parts of polyethylene glycol 6000 and one part of the CPT complexed form were dissolved in 50 ml ethanol by agitation. After drying at 45°C for 1 hour, the powder was preserved in a desiccator [4].

Study of CPT (free, encapsulated, or solid dispersion) and CPT 11 lactone ring stability in artificial media pH 2 and physiological serum

Five mg of camptothecin (free, encapsulated, or solid dispersion) and irinotecan were shaken for 72 hours in the dark in 500 ml of pH 2 buffer media and physiological media (saline). At appropriate time (1 H and 72 H), the solutions were filtered and quantified by HPLC. The analyses were carried out in triplicate.

Study of CPT (free, encapsulated, or solid dispersion) and CPT 11 lactone ring stability in human plasma

Investigations were performed with new blood. The blood was centrifuged at 6000 for 15 min at room temperature to isolate plasma and buffy coats. The plasma was supplanted by an isotonic arrangement (NaCl 0.9%). Then, plasma was treated with various arrangements at 0.01 mg/ml (v/v) (CPT, CPT11 and CPT binary and ternary systems). The samples were shaken for 72 hours in the dark. At the appropriate time (1 H and 72 H), the solutions were filtered and quantified by HPLC. The analyses were carried out in triplicate.

HPLC analyses

The analysis of lactone and carboxylate forms of CPT and CPT 11, were performed using HPLC-UV system (UltiMate 3000 RS-Variable Wavelength detector) which consisted of an auto-injector LC 1650, consisting of vacuum degasser, temperature-controlled well-plate autosampler, column thermostat, quaternary pump, and photodiode array detector set at 365 nm. Chromatographic analysis was performed using a Hypersil ODS C-18 (150 x 4.6 mm, 5 µm particle size, 80˚A pore size column) from Thermo (Bellefonte, PA, USA). The mobile phase consisted of a mixture of a borate buffer and acetonitrile (65: 35, v/v) with a flow rate of 1 ml/min, at a temperature of 30 °C and with an injection volume of 20 µl. Standard solutions were prepared by dissolving CPT or CPT 11 in a mixture of methanol/DMSO (95:5, v/v).

III. Results and discussion

Study of CPT and CPT 11 lactone ring stability in artificial media pH 2, physiological serum, and Human plasma

Chromatograms resulting from HPLC analysis of the lactone and carboxylate forms of CPT alone and CPT 11 solubilized in various media are shown in Figure 3.

The first stage of this work was to investigate the behavior of CPT and CPT 11 in various media: pH 2, saline and human plasma as a function of time (1 H and 72 H). Figure 4 summarizes the resulting data.

The findings show that camptothecin is stable in pH 2 media even after 72 H in solution. On the other hand, it is clear that in saline and human plasma, from the first hour, CPT is denatured and transformed into the inactive carboxylated form. Indeed, it is known that the lactone cycle of camptothecin is pH-dependent [14]. It was also observed that the CPT derivative (CPT 11) is stable regardless of the media and time of analysis, showing a small degradation.

The results are justified by those of FATMI et al. [4,5] for the encapsulation or/and solid dispersion of CPT not only to solubilize it but also to protect it during human administration.

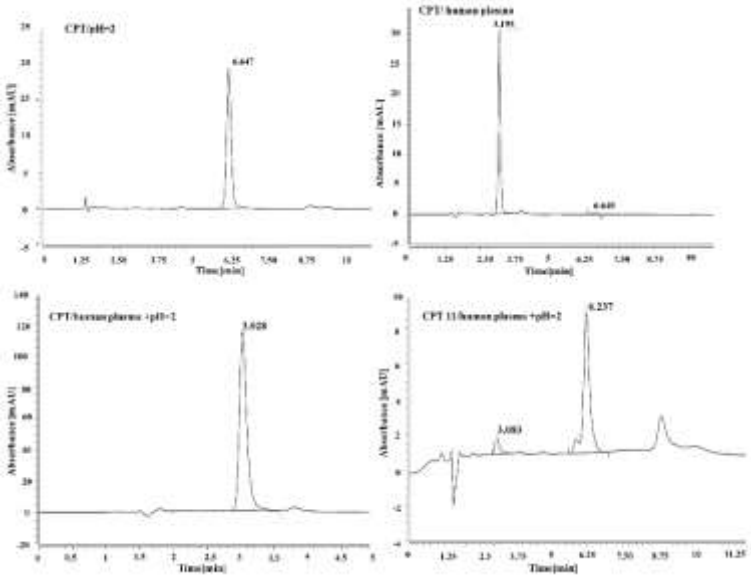

Figure 3: HPLC Chromatograms of the lactone and carboxylate forms of CPT alone and CPT 11 solubilized in various media

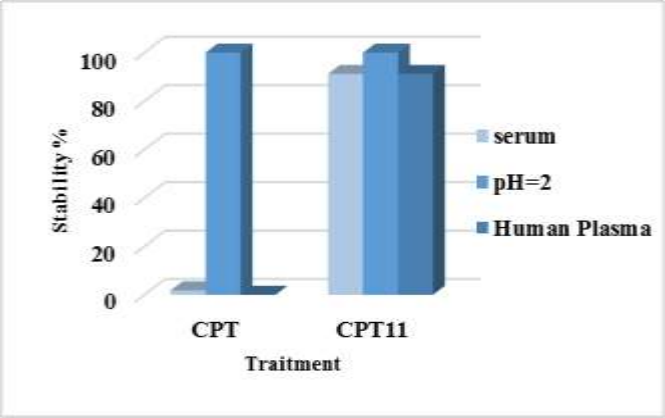

Figure 4: Stability of CPT and CPT 11 in the media: pH 2, serum and human plasma.

Study of CPT (alone, encapsulated or/and dispersed) and CPT 11 lactone ring stability in artificial media pH 2, physiological serum and Human plasma

Chromatograms resulting from HPLC analysis of the lactone and carboxylate forms of CPT (alone, encapsulated and in solid dispersion) and CPT 11 solubilized in various media are shown in Figure 5.

The results of the investigation of the behavior of CPT (alone, encapsulated or/and dispersed) and CPT 11 in various media: pH 2, saline and human plasma as a function of time (1 H and 72 H) are summarized in Figure 6.

All preparations were showing that camptothecin (alone, encapsulated or/and dispersed) is stable in pH 2 media even after 72 H in solution.

Unlike camptothecin alone, CPT in binary [CPT-β-CDs and CPT-PM-βCDs], and ternary [CPT/β-CDs/PEG 6000 and CPT/PM-βCDs/PEG 6000] systems showed protection from lactone ring degradation. This protection is maximal in CPT ternary systems for both types of cyclodextrins (βCD and PM-βCD). This protection is similar to the CPT derivative (irinotecan). It is probably due to the cavity stabilizing effect of CDs. J. Kang et al [15] in their research, had also established this protective capacity.

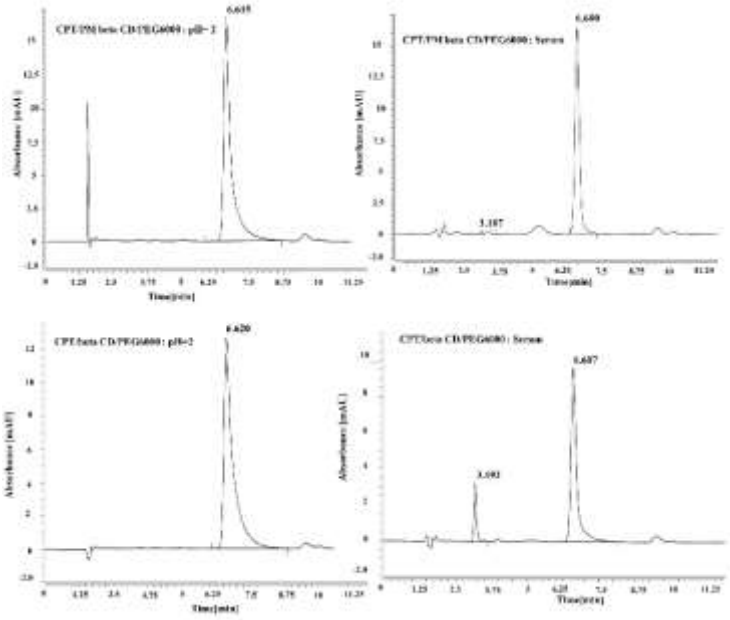

Figure 5: HPLC Chromatograms of the lactone and carboxylate forms of CPT (encapsulated and in solid dispersion) and CPT 11 solubilized in various media.

Among the binary systems, the PM-βCD-based system offers the best protection. This can be attributed to very polar methyl groups, which not only disrupt the intramolecular hydrogen bonding, making the PM-βCDs molecule highly soluble and stable but also extends the molecule cavity [15].

Also, ternary systems based on both types of CDs offer better percentages of protection (at an equal rate), this is probably due to the presence of PEG 6000 which is a very hydrophilic polymer and is currently widely used as a pharmaceutical carrier for the transport of poorly soluble and stable drugs. But also, it seems that this polymer cancels the effect of the external groups of the cyclodextrin cavity as reported by R. Sawant et al. [16].

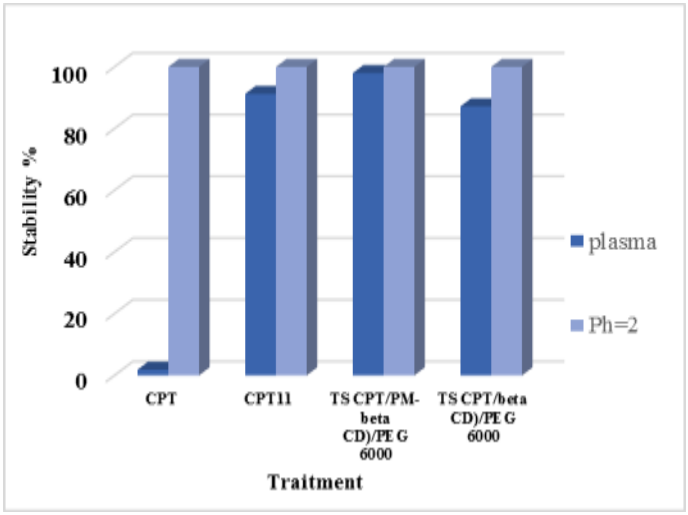

Figure 6: CPT (alone, encapsulated or/and dispersed) and CPT 11 in the media: pH 2, serum and human plasma.

Finally, the stability tests carried out on CPT alone, encapsulated and in solid dispersion in artificial media and human plasma demonstrate that the systems prepared by FATMI et al. [4,5] can lead to the protection of CPT lactone ring, essential to its therapeutic effect when administered in pharmaceutical form.

IV. Conclusions

To ensure the therapeutic effect of camptothecin, it is known that the integrity of the lactone cycle must be assured. Thus, the present work aims to study the chemical stability (lactone ring) of camptothecin (alone, encapsulated and in solid dispersion) while comparing it to that of a reference analog CPT 11. This stability is studied in pH 2, and physiological media and human plasma by high performance liquid chromatography (HPLC). Stability tests carried out on CPT alone, encapsulated and in solid dispersion in artificial media and Human plasma demonstrate that the systems particularly ternary systems [CPT/β-CDs/PEG 6000 and CPT/PM-βCDs/PEG 6000], can lead to the protection of CPT lactone ring in physiological media, and Human plasma for 72 hours. This protection is essential to the camptothecin therapeutic effect when administered in pharmaceutical form.

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Conflict of interest. The authors report no conflict of interest.

References

[1] L. Qi, Y. Zhang, W. Zhang, Y. Wang, Y. Han, Y. Ding. The inhibition of colorectal cancer growth by the natural product macrocarpal I, Free Radical Biology and Medicine. 16, 2383-391, 2021. https://doi.org/10.1016/j.freeradbiomed.2020.10.317.

[2] M. Ghanbari-Movahed, T. Kaceli, A. Mondal, M.H. Farzaei, A. Bishayee. Recent advances in improved anticancer efficacies of camptothecin nano-formulations: A Systematic Review, Biomedicines. 9, 480, 2021. https://doi.org/10.3390/biomedicines9050480.

[3] Q. Huang, X. Liu, H. Wang, X. Liu, Q. Zhang, K. Li, Y. Chen, Q. Zhu, Y. Shen, M. Sui. A nanotherapeutic strategy to overcome chemoresistance to irinotecan/7-ethyl-10-hydroxy-camptothecin in colorectal cancer, Acta Biomaterialia. 137, 262-275, 2022. https://doi.org/10.1016/j.actbio.2021.10.034.

[4] S. Fatmi, L. Bournine, M. Iguer-Ouada, M. Lahiani, F. Bouchal, M. Skiba. Amorphous solid dispersion studies of camptothecinñcyclodextrin inclusion complexes in PEG 6000. Acta Poloniae Pharmaceutica, 72(1), 179-92, 2015.

[5] S. Fatmi, L. Taouzinet, M. Lahiani-Skiba, M. Skiba, M. Iguer-Ouada. New formulation and evaluation of camptothecin encapsulated and/or dispersed suppository. Anti-Cancer Agents in Medicinal Chemistry. 21, 1183-1190, 2021. https://doi.org/10.2174/1871520620666200903150635.

[6] S. Saraf, A. Jain, A. Tiwari, A. Verma, S.K. Jain. Engineered liposomes bearing camptothecin analogue for tumour targeting: in vitro and ex-vivo studies. Journal of Liposome Research. 1(31), 326-341, 2020. https://doi.org/10.1080/08982104.2020.1801725.

[7] C.-S. Wu, D.-Y. Wu, S.-S. Wang. Heat-regulating polylactic acid/silica aerogel composite fabric: Preparation and characterization. Express Polymer Letter 16, 21-33, 2022. https://doi.org/10.3144/expresspolymlett.2022.3.

[8] A. Dodero, G. Schlatter, A. H´ebraud, S. Vicini, M. Castellano. Polymer-free cyclodextrin and natural polymer-cyclodextrin electrospun nanofibers: A comprehensive review on current applications and future perspectives. Carbohydrate Polymers 264, 118042, 2021. doi: 10.1016/j.carbpol.2021.118042.

[9] M. Petitjean, I.X. García-Zubiri, J.R. Isasi. History of cyclodextrin-based polymers in food and pharmacy: a review. Environmental Chemistry Letters 19, 3465-3476, 2021. https://doi.org/10.1007/s10311-021-01244-5.

[10] S. Alshehri, S.S. Imam, A. Hussain, M.A. Altamimi, N.K. Alruwaili, F. Alotaibi, A. Alanazi, F. Shakeel. Potential of solid dispersions to enhance solubility, bioavailability, and therapeutic efficacy of poorly water-soluble drugs: newer formulation techniques, current marketed scenario and patents. Drug Delivery 27, 1625-1643, 2020. https://doi.org/10.1080/10717544.2020.1846638.

[11] CFR - Code of Federal Regulations Title 21 Polyethylene glycol, Food and Drugs, Food and Drug Administration, indirect food additives: adjuvants, production aids, and sanitizers subpart d - certain adjuvants and production aids. https://www.accessdata.fda.gov

[12] A. Paudel, Z.A. Worku, J. Meeus, S. Guns, G. V. d. Mooter, Manufacturing of solid dispersions of poorly water-soluble drugs by spray drying: Formulation and process considerations. International Journal of Pharmaceutics 453(1), 253-284, 2012. DOI: 10.1016/j.ijpharm.2012.07.015

[13] X. Wang. Physico-chemical characterization of binary and ternary solid dispersions of itraconazole and indomethacin, Phd thesis, 2007.

[14] M.L. Bondì, R. Di Gesù, E.F. Craparo, Lipid Nanoparticles for Drug Targeting to the Brain, in: Methods in Enzymology, Elsevier, pp. 229-251, 2012. https://doi.org/10.1016/B978-0-12-391860-4.00012-4.

[15] J. Kang, V. Kumar, D. Yang, P.R. Chowdhury, R.J. Hohl. Cyclodextrin complexation: influence on the solubility, stability, and cytotoxicity of camptothecin, an antineoplastic agent. European Journal of Pharmaceutical Sciences 15, 163-170, 2002. https://doi.org/10.1016/S0928-0987,0100214-7.

[16] R. Sawant, R. Sawant, V. Torchilin. Mixed PEG–PE/vitamin E tumor-targeted immunomicelles as carriers for poorly soluble anti-cancer drugs: Improved drug solubilization and enhanced in vitro cytotoxicity. European Journal of Pharmaceutics and Biopharmaceutics 70, 51-57, 2008. https://doi.org/10.1016/j.ejpb.2008.04.016.