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Characterization and Molecular Modelling of Inclusion Complex between Hesperidin and β-cyclodextrin

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ABSTRACT: Hesperidin is a flavonone glycoside found in sweet orange and is effectively used in treatment for pain and disease. Although it plays a big role in the pharmaceutical area, hesperidin has a poor water soluble property. β-cyclodextrin (β-CD), a macrocyclic host with hydrophobic cavity and hydrophilic outer surface is a common pharmaceutical application to enhance the solubility, stability, safety and bioavailability of drug molecules. It can form inclusion complexes with many size-suitable guest molecules due to their unique molecular structure. In this study, the effect of β-CD on the aqueous solubility of hesperidin was investigated. The complex of β-CD-hesperidin was prepared via modified kneading method and the formation of complex was confirmed using FTIR, DSC, UV-VIS and NMR analysis. The structure was simulated using Gaussian 2003 and Hyperchem 7.5 softwares. It was predicted that hesperidin is stated exactly in the middle of the cavity of β-CD where both twin ring fully immersed in the center of the cavity facing the interior surface. While another two ring facing downward to the wider side.

Keywords: Hesperidin, β-cyclodextrin, Inclusion complex.

INTRODUCTION

Over the years, the formulation of poorly water soluble compounds presented interesting challenges for scientist in the pharmaceutical industry. [1]. Nearly one-third of drugs in drug development are water insoluble and one half in trials because of under privileged pharmacokinetics. [2]. These poor water soluble drugs are allied with slow drug absorption leading to inadequate and variable bioavailability and gastrointestinal mucosal toxicity [3]. Several techniques have been reported in the literatures that were used to improve the solubility, dissolution rate and bioavailability of insoluble drugs. One interesting technique is via the cyclodextrin approach. [4].

Cyclodextrin (Figure 1) has been extensively developed in order to increase solubility, enhance bioavailability, improve stability, masking of bad taste or odour, reduce volatility, transform liquid or gas into solid form, reduce side effects and possibility of a drug release system . [5]. Pharmaceutical applications of cyclodextrins as additive and drug complexing agents have been growing rapidly, as reflected in the increasing number of medicinal products being placed on the markets as cyclodextrin based formulations . [6]. Complexation between cyclodextrin and the drugs are determined either by hydrophobic interactions, hydrogen bonds, van der waals interactions, conformational energy, dipole-dipole and ion-dipole interactions and the rearrangement of water molecules originally surrounding both cyclodextrin and the guest molecule. [7]. Out of the three parent cyclodextrins, β-cyclodextrin (β-CD) appears more useful as a pharmaceutical agent because of its complexing ability, cavity dimension, low cost and higher productive rate. Its' cavity size is suitable for common pharmaceutical drugs with molecular weight between 200-800gmol^{-1}. [4]

Figure 1: Structure of β-cyclodextrin (top view)

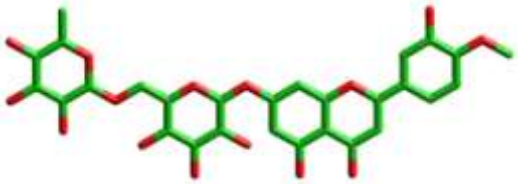

Figure 2: Hesperidin

Hesperidin (hesperetin 7-rutinoside) (Figure 2) is a flavanone glycoside, abundantly found in citrus fruits (family Rutaceae). [8]. It has shown to possess an antioxidant effect [9], blood lipid lowering [10] and anti-carcinogenic activities [11]. However, the oral bioavailability of hesperidin is limited. Most probably due to its crystalline state, this flavanoid is slightly soluble in water (57mg/L), a characteristic which leads to a very low dissolution rate and an irregular absorption of the drug from oral solid dosage form in the gastrointestinal tract. [12]. Furthemore, hesperidin also possess poor transmembrane permeability and is believed to be absorbed primarily by the paracellular pathway. [13].

In this study, investigations were performed on the possibility of complexation of hesperidin with β-CD for improving the solubility, thereby increasing the bioavailability and therapeutic efficacy of the drug.

MATERIALS AND METHODS

Research Materials

Hesperidin, MW: 610.6 (Santa Cruz Biotechnology) and β-Cyclodextrin, MW: 1134 (Sigma Aldrich).

Preparation of Sample

The preparation of solid complex between hesperidin and β-CD were performed in a 1:1 ratio using modified kneading method. The β-CD was crushed into a mortar until become powdery form. Hesperidin was then introduced to the powdered β-cyclodextrin. The mixture was rotated in one direction to ensure that the mixed powder was crushed and well mixed together. The pulverization process took place approximately one hour. Next, ten drops of distilled water was added dropwise and mixed in the mortar until it became paste like. Lastly, the sample was stored and labelled in a borosilicate glass and the sample was freeze dried.

Spectral Investigation and Characterizations

An IR spectrophotometer (Thermo Scientific Nicolet 6700 FT-IR spectrometer) was used for the IR analysis. All spectras were recorded within a range of 4000–500 cm^{-1}.OMNIC software was used to show all the spectras. DSC

analysis was performed using a TA Instruments SDT-Q600 Simultaneous TGA /DSC under a nitrogen purge (20 ml/min). A heating rate of 10°C/min was employed using temperature ranges from 25 to 500°C. The UV/VIS spectrophotometer (Perkin Elmer Lambda 25) used in wavelength ranges from 200nm to 400nm. NMR spectras were recorded at 24°C on a Bruker DRX 400- AVANCE spectrometer operating at 500 MHz, equipped with a 5 mm inverse probe with z-gradient coil. D_2O or DMSO were used as solvent for analysis.

Molecular Modelling Studies

The starting geometries of hesperidin (guest molecule) and β-cyclodextrin (host molecule) were constructed based on the structures that generated from crystallographic parameters provided by the Cambridge Structural Database (CSD). [14]. Then, these molecules were optimized separately using semi-empirical pm3 method of Gaussian 2003 software [15] before constructed their complexes using Hyperchem 7.5 software [16] and simulated in vacuum condition. [17].

RESULTS AND DISCUSSION

FTIR (Fourier Transform Infrared) Spectroscopy

IR spectrum of β-CD shows a large band between 3000-3700 cm^{-1} (OH_{str}), 2921 cm^{-1} ($CH_{aliphatic}$), 1646 cm^{-1} ($C=O_{str}$), 1417 cm^{-1} ($C=C_{aromatic}$). IR spectrum of hesperidin is identified by absorption peaks at 3403 and 3534 cm^{-1} (OH_{str}), 2917 cm^{-1} ($CH_{aliphatic}$), 1642, 1597 and 1519 cm^{-1} ($C=C_{aromatic}$). It is clearly seen that the main peaks of both molecules were shifted in complex spectrum and it suggested that complexation was formed.

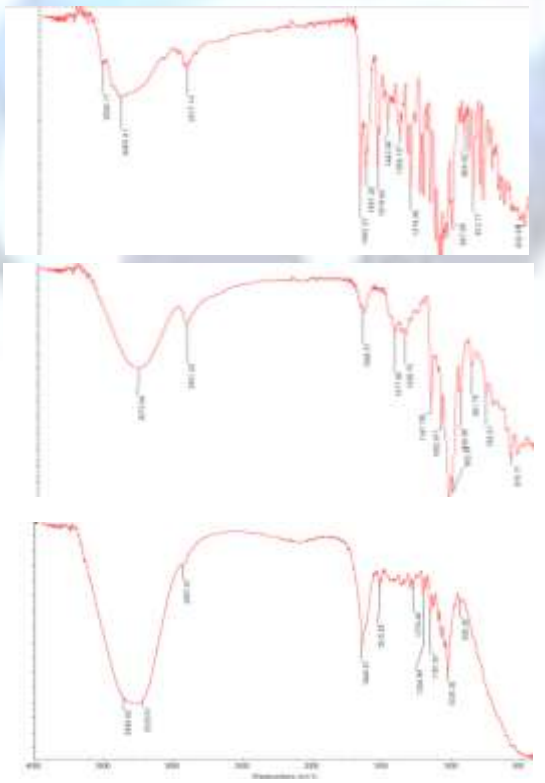

Figure 3: FTIR spectra of free hesperidin, free β-CD and complexed hesperidin- β-CD

DSC (Differential Scanning Calorimetry)

Generally, the melting point of complexes will change (decrease or disappear) to a different temperature and the intensity from their pure compound in the crystal lattice. [4]. The DSC thermograms for the complex shows the persistance of the endothermic phenomenon due to loss of water and the melting peak described for the drug slightly shifted to the higher temperature. A new endothermic peak was observed with T_{onset} 246°C. This new peak is not attribute to the degradation because no weight loss was observed at this temperature. The curve showed different thermal stabilities and the degradation was occured at high temperature than that for the free hesperidin.

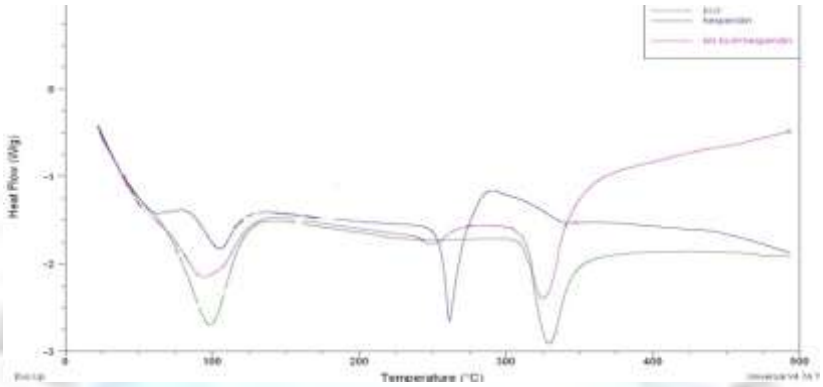

Figure 4: DSC thermograms of free hesperidin, free β-CD and complexed hesperidin- β-CD

Ultraviolet-Visible (UV/VIS) Spectrophotometer

The existence of aromatic in the inclusion complex between the β-CD with hesperidin was confirmed by the UV-VIS spectrophotometer analysis. Complexation causes a change in the absorption spectrum of hesperidin molecule. During the spectral changes, the chromophore of the hesperidin is transferred from an aqueous medium to the non-polar cyclodextrin. These changes must be due to a perturbation of the electronic energy levels of the guest caused either by direct interaction with the cyclodextrin, by the exclusion of solvating water molecules or by a combination of these two effects. Small shifts are observed on the UV spectra of the included hesperidin.

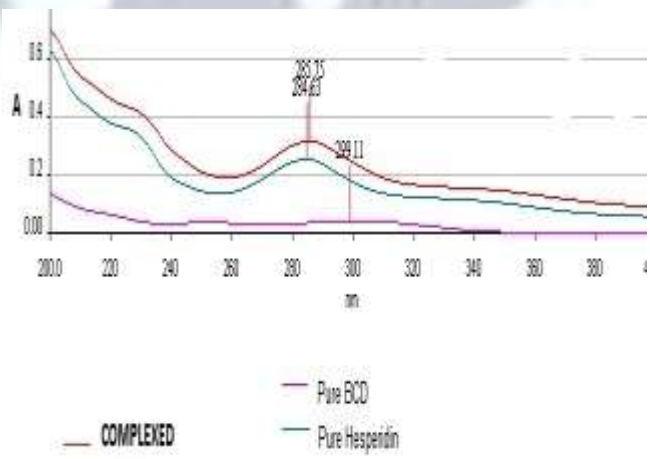

Figure 5: UV-VIS spectra of free hesperidin, free β-CD and complexed hesperidin, β-CD

H-NMR (H-Nuclear Magnetic Resonance Spectroscopy)

The most evidence for the inclusion of guest into a cyclodextrin cavity in solution is obtained by ^{1}H-NMR spectroscopy. ^{1}H-NMR may also be used to determine the direction of penetration of guest molecules into the cyclodextrin cavity. [13]. Comparing the spectrum of free β-CD and its complex, it was found that all the shifts were shifted upfield ($\Delta\delta_1$ 0.472, $\Delta\delta_2$ 0.476, $\Delta\delta_3$ 0.455, $\Delta\delta_4$ 0.461 and $\Delta\delta_5$ 0.462) and thus suggested that inclusion does take place.

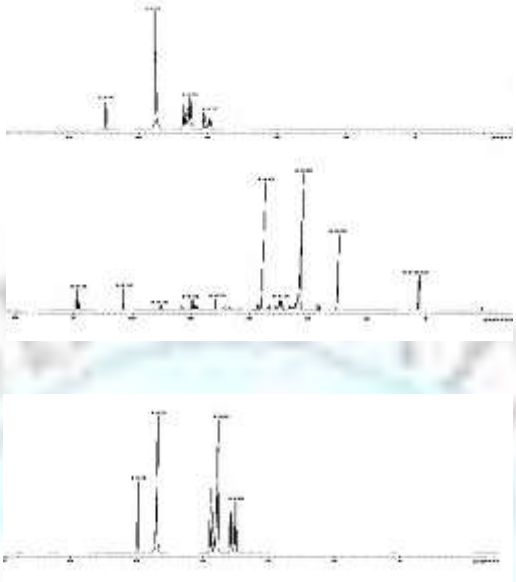

Figure 6: NMR spectra of free β-CD, free hesperidin and complexed hesperidin- β-CD

Molecular Modelling Studies

The inclusion complexes were simulated using Gaussian 2003 and Hyperchem 7.5 have predicted that the guest molecule (hesperidin) is stated exactly in the middle of the cavity where both twin ring fully immersed in the center of the cavity facing the interior surface. While another two ring facing downward to the wider side (Figure 7). This position would simulate the hydrogen bonds between both molecules. The interaction allows this complexation to be soluble in water. It was predicted that two hydrogen bonds were formed between hesperidin and β-CD, one hydrogen bond within the hesperidin molecule and six hydrogen bonds within the β-CD molecule (Figure 8).

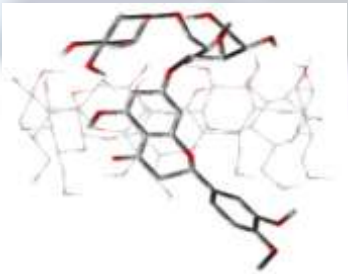

Figure 7. The most stable predicted complex between β-cyclodextrin and hesperidin.

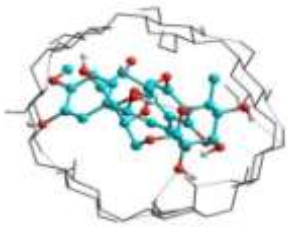

Figure 8: The hydrogen bond (inner and outer) formed between β-cyclodextrin and hesperidin

CONCLUSION

From the results of this study, it can be suggested that hesperidin solubility can be improved by inclusion complex with β-cyclodextrin. The thermal stability, infrared and absorption shown proven that there are interactions between the drug and the host. Thus, suggested that complexation has occurred. Due to the significant changes in the spectras of IR, DSC, UV-Vis and H-NMR analysis and supporting data from molecular modelling studies, it can be concluded that hesperidin does complex with β-cyclodextrin. It is expected that the solubility of hesperidin in water will be improved via complexation and consequently solved the problem in the pharmaceutical industry.

ACKNOWLEDGEMENT

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REFERENCES

[1]. Patel, N.D, Patel, K.V, Panchal, L.A, Shukla, A.K & Shelat P.K. (2011). An Emerging technique for poorly Soluble Drugs: Self Emulsifying Drug Delivery System. Review Article. International Journal of Pharmaceutical and Biological Archive, 2(2) p. 621 -629.

[2]. Lobenberg, R. & Amidon, G.L. (2000). Modern Bio-availability, bio-equivalence and biopharmaceutics classification system; new scientific approaches to international regulatory standards. Europe Journal of Pharmaceutical and Biopharmaceutical, 50, p. 3-12.

[3]. Patil, J.S., Kadam, D.V., Marapur, S.C. & Kamalpur, M.V. (2010). Inclusion complex system; a novel technique to improve the solubility and bioavailability of poorly soluble druds: a review. International Journal of Pharmaceutical Sciences Review and Research., 2(2), p. 29-34.

[4]. Doile, M.M., Fortunato, K. K., Schmücker, I.C., Schucko, S. K., Silva, S.S.M. & Rodrigues P.O. (2008). Physiochemical properties of dissolution studies of dexamethasone acetate-β-CDinclusion complexes produced by different methods. AAPS PharmSciTech., 9, p. 314-320.

[5]. Reddy, M.N., Rehana, T., Ramakhrisna, S., Chowdry, K.P.R. & Diwan, P.V. (2004). B-CDcomplexes of celecobix: molecular modeling, characterization, and dissolution studies. AAPS PharmSci., 6(7), p. 314-321.

[6]. Calabrò, M.L., Tommasini, S., Donato, P., Raneri, D., Stancanelli, R., Ficarra, P., Ficarra, R., Costa, C., Catania, S., Rustichelli, C. & Gamberini, G. (2004). Effects of α and β-CDcomplexation on the physico-chemical properties and anti-oxidant activity of some 3-hydroxyflavones. J. Pharm. Biomed. Anal., 35, p 365-377.

[7]. Grillo, R., Silva Melo, N.F., Moraes, C.M., Rosa, A.H., Roveda, J.A.F., Menezes, C.M.S., Ferreira,E.I. & Fraceto, L.F.(2007). Hydroxymethylnitrofurazone : Dimethyl-β-CDInclusion Complex: A Physical Chemistry Characterization. J.Biol. Phys., 33, p 445-453.

[8]. Majumdar, S. & Srirangam, R. (2009). Solubility, stability, physiochemical characteristics and in vitro ocular tissues permeability of hesperidin : a natural bioflavanoid. Pharm. Res., 26 (5), p 1217-1225.

[9]. Bouskela,E., Cyrino,F.Z. & Lerond, L.(1997). Effects of Oral Administration of Different Doses of Purified Micronized Flavanoid Fraction on Microvascular Reactivity after Ischaemia/Reperfusion in the Hamster Cheek Pouch. Br. J. Pharmacol., 122, p 1611-1616.

[10]. Borradaile, N.M., Carroll, K.K. & Kurowska, E.M. (1999). Regulation of HepG2 cell Apolipoprotein B Metabolism by the Citrus Flavanones Hesperetin and Narigenin. Lipids, 34, p 591-598.

[11]. Tanaka,T., Makita, H., Kawabata, K., Mori,H., Kakumoto, M., Satoh,K., Hara, A., Sumida, T., Tanaka, T. & Ogawa, H. (1997). Modulation of N-Methyl-N Amylnitrosamine-induced Rat Oesophageal Tumourigenesis by Dietary Feeding of Diosmin and Hesperidin, Both Alone and in Combination. Carcinogenesis, 18, p 957- 965.

[12]. Sansone,F., Rossi,A., Del Gaudio,P., De Simone,F., Aquino, R.P. & Lauro, M. (2009). Hesperidin Gastroresistant Microparticles by Spray-Drying: Preparation, Characterization and Dissolution Profiles. AAPS PharmSciTech, 10(2), p 391-401.

[13]. Singh, R., Bharti, N., Jyotsana, M. & Hiremath, S.N. (2010). Characterization of cyclodextrin inclusion complexes-a review. Journal of Pharmaceutical Science and Technology, 2(3), p. 171-183.

[14]. Seidel, R. W., & Koleva, B. B. (2009). -Cyclodextrin 10.41-hydrate. Acta Crystallographica Section E: Structure Reports Online, 65(12), o3162-o3163.

[15]. Honda, Y., Kitao, O., Nakai, H., Klene, M., Li, X., Knox, Jaramillo, J. (2003). Gaussian 03, revision B. 04; Gaussian. Inc.: Pittsburgh, PA.

[16]. Release, H. (2002). 7.5 for windows, molecular modeling system, Hypercube. Inc. http://www. hyper. com.

[17]. Mokhtar, A. & Adnan, R. (2012). Preparation method and characterization of inclusion complex of theophylline.beta cyclodextrin. ESTEEM Academic Journal, 8 (2), p. 50-63.