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Abstract

Modulation of Oleanolic Acid Dissolution Profile via Solid State Manipulation and Self-Nanoemulsifying Drug Delivery Systems (SNEDDS)

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Oleanolic acid (OA), a naturally occurring pentacyclic triterpenoid, is a biologically active marker compound commonly present in Chinese herbs, such as *Fructus Ligustri Lucidi*, *Fructus Forsythiae* and *Radix Ginseng* etc. Oleanolic acid has been successfully used as an OTC oral drug to treat human liver disorders in China due to its hepatoprotective effect. In addition, it has been shown to exhibit anti-inflammatory, antitumor and anti-hyperlipidemic activities. Up to now, the commonly used formulations of OA in the market are tablets, capsules, and pills.

However, being hydrophobic, OA exhibits poor aqueous solubility. During in-vitro dissolution study, less than 1 μg/ml OA were dissolved from raw solid form into an aqueous buffer (pH 1 or 7) after 2 hours. Furthermore, given the low Caco-2 cell permeability and extensive hepatic microsomal metabolism, the low oral bioavailability of oleanolic acid may be due to a poor gastrointestinal absorption and extensive hepatic first-pass metabolism.

In order to modify the in vitro dissolution of oleanolic acid, two approaches are utilized in this study. One is to prepare oleanolic acid solvates through recrystallization method, while the other is to prepare its self-emulsifying nanoemulsion system (SNEDDS). Furthermore, the permeability of OA raw material and its nanoemulsion were determined on the Caco-2 cell monolayer model.

The solid state properties of oleanolic acid recrystallized from a variety of solvents were investigated. Glassy materials were prepared with dichloromethane and

chloroform solvents in both cold crystallization and solvent evaporation. Crystalline forms of OA, including a methanol solvate, an ethanol solvate and an acetone non-solvate, were prepared by dissolving its raw material into different organic solvents and evaporating organic solvents at room temperature in a dark place for several weeks. The filtered crystals were carefully dried and stored under ambient conditions and then characterized. Upon desolvation, both the methanol and ethanol solvates were found to undergo phase transformation to a crystalline phase similar to OA-Acetone around 190-195°C. The PXRD patterns of commercial pharmaceutical grade OA and the OA-Methanol were similar, so the commercial form is probably desolvated oleanolic acid methanol solvate. Specific surface area of the three crystalline materials was determined by BET nitrogen adsorption. HPLC was utilized to quantify the in vitro dissolution of OA raw material (OA-RW), solvates, non-solvate and commercial available tablet. Dissolution studies showed that ~89.5% of OA could be released from the raw material in 1% SDS (pH 7.0) after 24 hours, which is higher than those of the crystalline forms (<70%). Both of the initial and intrinsic dissolution rates of OA-RW were obviously faster than those of the other forms which may due to its higher specific surface area. For the other crystalline forms, methanol and ethanol solvates showed higher intrinsic dissolution rates than the acetone non-solvate because of the favorable reduction in free energy through the mixing of methanol/ethanol with water.

Meanwhile, a self-nanoemulsified drug delivery system (SNEDDS) of oleanolic acid (OA) for oral delivery is also prepared in this study. Solubility of OA under different systems was determined for excipient selection purpose. Three formulations, where OA was fixed at the concentration of 20mg/g, were prepared utilizing Sefsol 218 as oil phase, Cremophor EL and Labrasol as primary surfactants, and Transcutol P as cosurfactant. Pseudo-ternary phase diagrams were constructed to identify self-emulsification regions for rational design of SNEDDS formulations. Sefsol 218 was found to provide the highest solubility among all medium-chained oils screened. Efficient self-emulsification was observed for the systems composing of Cremophor EL and Labrasol. The surfactant to cosurfactant ratio greatly affected the droplet size of the nanoemulsion. Based on the results in dissolution profiles, stability data and particle size distribution, three optimized formulations were selected: Sefsol 218:Cremophor EL:Labrasol:Transcutol P (50%:17.5%:17.5%:15%, w/w), Sefsol

218:Cremophor EL:Labrasol:Transcutol P (50:20:20:10%, w/w), and Sefsol 218:Cremophor EL:Labrasol (50:25:25% w/w). More than 90% and 60% OA could be rapidly released from the nanoemulsions in water and simulated gastric fluid within 10min, respectively. In contrast, none of the OA could be detected in dissolution medium from the OA raw material and its commercial available tablet, where the equilibrated concentration was below the detection limit of HPLC (1 μg/ml). Three formulations were stable at room and subambient temperature (e.g. 4°C) for at least six months. However, they are sensitive at higher temperature (e.g. 40°C). These results suggested the potential use of SNEDDS to improve solubility and dissolution for poorly water soluble triterpenoids such as OA.

The Caco-2 cell monolayer, the well-studied model for assessing drug absorption, was used to evaluate the permeation rates of oleanolic acid in both apical to basolateral and basolateral to apical directions. P_{app} values of oleanolic acid or its SNEDDS formulation (4 μg/ml OA) were all lower than 1×10^{-6} cm/s, from both directions, suggesting that poor intestinal permeability maybe the rate limiting step for its oral absorption.

Above results indicate that slower dissolution rate of OA can be engineered by the formation of OA crystalline materials, which can be potentially useful for OA sustained release products. And because that OA could be rapidly and completely released from its nanoemulsion system, it was suggested that SEDDS of OA could be developed into immediate-release products. However, due to the limited intestinal permeability of OA, more in vivo animal work needs to be performed to verify whether the oral bioavailability of OA could be enhanced by formulated as SNEDDS.

Keywords: Oleanolic acid; Recrystallization; Solvate; SNEDDS; Dissolution; Caco-2 cell absorption model