International Journal of Pharmaceutics 369 (2009) 5–11

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Studies on coumestrol/β-cyclodextrin association: Inclusion complex characterization

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ARTICLE INFO

Article history:
Received 2 July 2008
Received in revised form 10 October 2008
Accepted 13 October 2008
Available online 5 November 2008

Keywords:
Coumestrol
Cyclodextrin
Complexation
Modeling

ABSTRACT

Coumestrol is an estrogenic and antioxidant agent, characterized by its low solubility in aqueous and lipophilic media, once in the aglicone form. In order to improve its solubility in water, coumestrol was associated with β-cyclodextrin in aqueous media followed by freeze-drying and characterized by SEM, ^{1}H NMR and molecular modeling. The analysis proved the existence of an inclusion complex, with higher probability of inclusion of the coumestrol B-ring into the wider rim of the β-cyclodextrin molecule.

1. Introduction

Coumestrol belongs to the group of coumestans, included in the Leguminosae family. It is structurally similar to isoflavones and constitutes a fully oxidized version of pterocarpans (Al-Maharik and Botting, 2004; Ganry, 2005). Its molecular structure is presented in Fig. 1.

This compound is found in clovers and alfalfa sprouts (Al-Maharik and Botting, 2004; Ganry, 2005) and has been receiving attention due to its estrogenic and antioxidant properties. Coumestrol acts on ERα and ERβ, estrogen receptors, having seven times more affinity for ERβ than for ERα (Benassayag et al., 2002; Garey et al., 2001). These receptors are present in the epidermis (queratinocites, Langerhans cells and melanocites), blood vessels and dermis (fibroblasts), among other places of the human body (Birt et al., 2001; Krazeisen et al., 2001; Pocock et al., 2002; Lapcik et al., 2003; Thornton et al., 2003; Sator et al., 2004). Coumestrol is the most potent phytoestrogen and competes with zearalenol and genistein for the 17β-estradiol receptors binding, depending on which receptor it acts (ERα or ERβ) (Benassayag et al., 2002).

Besides its estrogenic activity, coumestrol acts as an antioxidant due to its capacity of donating electrons from the hydroxyl groups present in the A and B-rings; removing free radical and preventing oxidative damages (Mitchell et al., 1998). Mitchell et al. (1998) reported that coumestans can inhibit peroxidation reactions sixteen times more than α-tocoferol, a natural antioxidant present in the membranes. More recently, Georgetti et al. (2003) and Lee et al. (2006) demonstrated that the red clover extract (*Trifolium pratense* L.), which contains coumestrol, presents a high antioxidant activity, and that the pterocarpans from roots of *Glycine max* L. have potent low-density lipoprotein (LDL) oxidation inhibitory activity, showing that coumestrol is 20 times more antioxidant than genistein and daidzein.

Taken together, coumestrol seems to be a promising agent for skin aging prevention, especially for post-menopausal women. However, its activities are conditioned to the aglicone form, which presents reduced solubility in organic solvents and in hydrophilic vehicles (Budavari, 2001; Silva et al., 2001; Havsteen, 2002). This fact impairs the development of a topical pharmaceutical dosage form. In order to improve the solubility of the aglicone form in water, and therefore to facilitate its delivery to the skin as well as its incorporation into a hydrophilic vehicle, the association of coumestrol with cyclodextrins seems to be a promising strategy. In fact, the association of coumestrol with a cyclodextrin was for the first time investigated by Cannavà et al. (2008), who performed phase-solubility studies and employed FTIR-ATR analysis to point out the implication of particular functional groups of coumestrol in the inclusion complexes with β-cyclodextrin and hydroxypropyl-

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doi:10.1016/j.ijpharm.2008.10.026

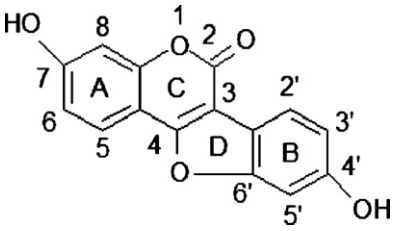

Fig. 1. Chemical structure of coumestrol.

β-cyclodextrin prepared by the co-precipitation method. In the present study, a solid coumestrol:β-cyclodextrin complex was prepared by freeze-drying method. The morphology of the complex was characterized by scanning electron microscopy (SEM) and the spatial configuration of this association was firstly proposed by means of ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) and molecular mechanics calculation. β-Cyclodextrin was chosen due to its adequate cavity size, which enables the formation of inclusion complexes with many substances, as well as to its accessible cost and related advantages concerning the industrial feasibility of a topical dosage form containing coumestrol (Loftsson and Brewster, 1996; Singh et al., 2002; Del Valle, 2004; Yan et al., 2006).

2. Materials and methods

2.1. Materials

Coumestrol, code 27885 (95% of purity), was purchased from Sigma–Aldrich (São Paulo, Brazil) and β-cyclodextrin was kindly donated by Roquette et Frères (France). Dimethyl sulfoxide d_6 was purchased from Tedia (Rio de Janeiro, Brazil) and potassium bromide from Synth (Porto Alegre, Brazil). All other reagents and solvents used were of analytical grade.

2.2. Preparation of coumestrol associations

The complex preparation followed the procedure reported by Higuchi and Connors (1965). An excess amount of coumestrol (1.5 mg) was added to a vial containing 2.5 ml of either water or a β-cyclodextrin solution (considering a 1:1 molar ratio). These dispersions were stirred in a water bath (IKA®-Werke EH4 Basic) at 37 °C, during 48 h. After this period, the dispersions were cooled down to room temperature, filtered through a 0.45 μm pore diameter membrane to volumetric flasks of 5 ml, and the volume was made up with water. An aliquot (1 ml) of this solution was transferred to other 5 ml volumetric flasks and diluted with methanol for drug assay by ultraviolet spectrophotometry at 343 nm (Hewlett Packard 8452A—Diode Array Spectrophotometer) over the concentration range of 1–5 μg/ml of coumestrol ($R^2 > 0.999$), following a previously validated method based on ICH guidelines (2005). The solution (with theoretical 1:1 drug:cyclodextrin content), was freeze-dried (Edwards Modulyo 4K, −60 °C, under light protection) and stored for further analysis and drug content evaluation.

2.3. Characterization of coumestrol associations

The scanning electron microscopy (SEM) was performed to the raw materials and to coumestrol: β-cyclodextrin freeze-dried product at three magnifications (2000×, 1000× and 500×), using the Jeol 6060 apparatus, after samples had been gold sputtered. The parameters used were: SEI mode, samples height less than 5 mm; voltage of 20 kV; WD of 11 mm, spotsize of 40 and LC of 60 μA.

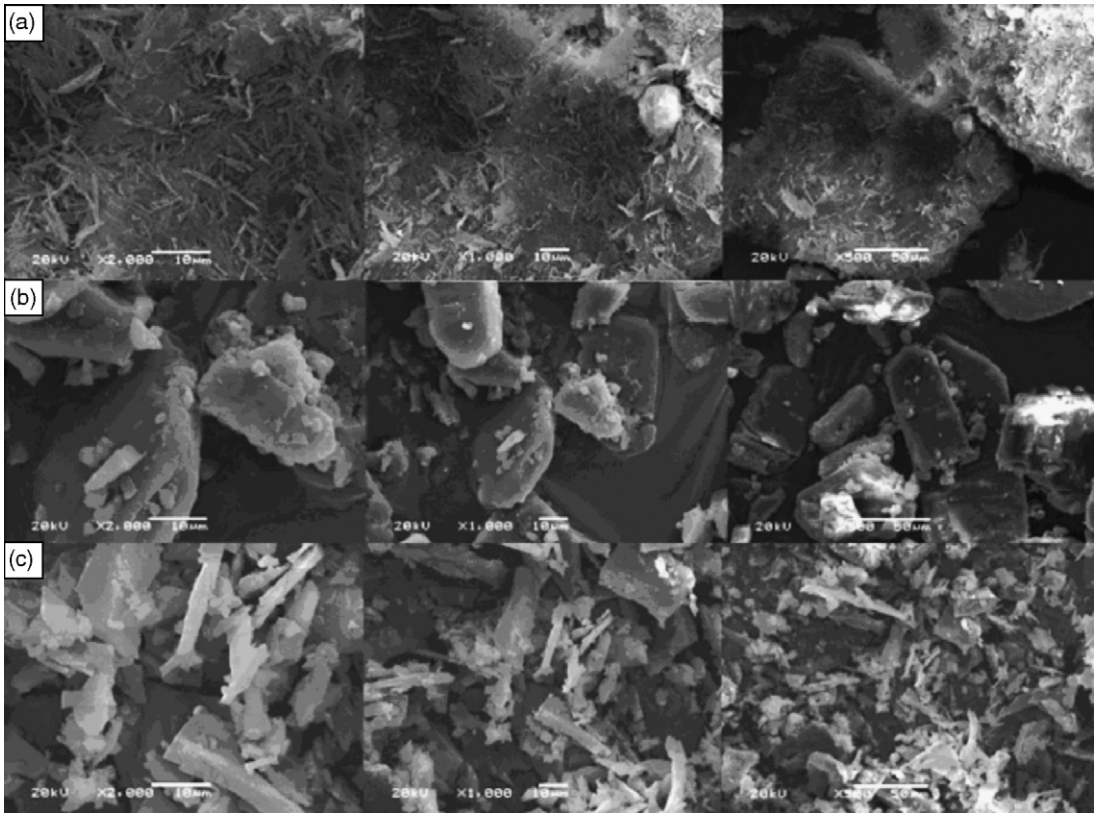

Fig. 2. SEM of (a) coumestrol, (b) β-cyclodextrin and (c) coumestrol:β-cyclodextrin complex (magnifications of 2000×, 1000× and 500×, respectively).