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Alginate-Based Hydrogel Containing Minoxidil/ Hydroxypropyl-β-Cyclodextrin Inclusion Complex for Topical Alopecia Treatment

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ABSTRACT

Cutaneous minoxidil (MXD) formulations were developed with the intent to reduce the side effects of the cosolvents propylene glycol and ethanol, frequently used in commercial MXD solutions. Completely aqueous alginate-based hydrogels were investigated and MXD aqueous solubility was improved using inclusion complexes with hydroxypropyl-β-cyclodextrin (HP-β-CD) at 2 different molar substitution degree (MS), namely 0.65 and 0.85. HP-β-CD MS 0.65 was selected for its improved solubilizing ability toward MXD. At concentration of 39% w/v, this cyclodextrin increased the intrinsic aqueous solubility of MXD of about 22-fold. The calculated complexation constant was 2309 ± 20 M^{-1}, and the inclusion process was spontaneous and enthalpically driven. Nuclear magnetic resonance studies (Job plot, ^1H, 2D correlations spectroscopy, nuclear overhauser effect spectroscopy, and rotating-frame overhauser enhancement spectroscopy) confirmed the stoichiometry 1:1 between MXD and HP-β-CD providing information about the exact geometry of the inclusion complex. Rheological and *in vitro* release studies performed on the formulation loaded with MXD 3.5% w/w proved that the inclusion complex increased the viscosity of the hydrogel modulating the release of the free drug. Furthermore, the hydrogel formulation facilitate MXD to permeate into the skin and did not damage epidermis, suggesting that these completely aqueous MXD delivery systems can be proposed as alternative formulations to commercial solutions.

Introduction

Androgenetic alopecia (AGA) is the most common hair-loss disorder affecting men and women especially with the increasing of the age[1,2] This disorder transforms large terminal follicles into small miniaturized ones,[3] leading to a gradual decrease in the density of hairs and simultaneous increase of vellus hairs[4] which can affect the entire scalp (alopecia totalis) or the temporal-frontal loss in men and central thinning in women.[1] Even if the reasons of these changes have not been clearly recognized, male pattern baldness is known to be due to androgens, in particular to the dihydrotestosterone.[5] Hair loss often has a severe social and emotional impact and in many cases, it can be a self-limiting condition. Therefore, many patients require an efficient treatment to solve this problem.

Minoxidil (MXD) is a pyrimidine derivate drug widely used for the topical treatment of AGA. MXD may stimulate hair growth by increasing the anagen phase of the hair cycle, but the exact mechanisms are still unclear.[6] Owing to its limited water solubility, MXD topical formulations must contain high percentage of cosolvents such as ethanol and propylene glycol that could significantly affect its solubility and drug penetration through the skin.[7] Many commercial products containing MXD (from 1% to 5% w/w) are solution constituted of water and the cosolvents ethanol/propylene glycol. For example, Rogaine® extra strength (5% w/v MXD)

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contains 30% and 50% w/v of ethanol and propylene glycol, respectively. In addition, the galenic solution of MXD described in the British Pharmacopeia is based on the same excipients. These formulations are not sustained drug delivery systems and thus the patient should apply the preparation at least twice a day to ensure the pharmacological effect.[8] However, after the application of such formulations on the skin, some patients present clinical complaints like pruritus and scaling of the scalp,[9] mainly due to the propylene glycol. In added, ethanol quickly evaporates after spreading over the bold skin, whereas, the greasy propylene glycol/water mixture stays on the applied area causing allergic responses, dandruff, dermatitis, rash and itchiness and therefore the patients often suspend the treatment. Consequently, new organic solvent−free topical formulations are required to decrease adverse effects and optimize AGA treatment. An alternative preparation could be a hydrogel, a completely aqueous formulation capable to realize a slow release of MXD.[10] Several homo-polymers or copolymers form hydrophilic gel networks, and among them alginate can be an interesting substance. Alginate is an anionic copolymer composed of residues of α-D-mannuronic acid and R-L-guluronic acid and characterized by biocompatibility and biodegradability.[11] Moreover, this polymer helps to repair wounds,[12] which can be useful to rebalance the compromised scalp in patients with alopecia.

However, to realize this kind of formulation, it is mandatory to improve the aqueous solubility of MXD. This target can be reached using cyclodextrins (CDs), which are well known to form inclusion complexes with many hydrophobic guest drugs improving their solubility and dissolution profile in water.[13-15] The benefits of a drug complex with CDs also include enhanced chemical and physical drug stability,[16] and absorption.[17]

The challenge to form inclusion complexes between different CDs (β-ciclodextrin, β-CD, hydroxypropyl-β-cyclodextrin (HP-β-CD), methyl-β-cycodextrin, Me-β-CD) and MXD was reported in different studies.[10,18-20] Among them, the HP-β-CD is particularly interesting because it forms a good inclusion complex with MXD,[20] and it has higher aqueous solubility than β-CD and is cheaper than Me-β-CD. In addition, HP-β-CD can extract cholesterol from cells with lower capacity than the Me-β-CD, therefore it is less cytotoxic.[21] Finally, the monograph of HP-β-CD is present in the European and the United States Pharmacopoeias (EuPh ninth, USP40-NF35).

Nevertheless, a comprehensive study on inclusion complex between HP-β-CD and MXD has not been conducted until now, and nobody has evaluated if HP-β-CDs with different molar substitution degree (MS, indicating the average number of hydroxypropyl substituents that have reacted with 1 glucopyranose repeat unit) could form different inclusion complexes with MXD. In fact, as previously reported in the literature,[21] the solubilizing effect of HP-β-CD for other tested drugs depends on its average MS. Furthermore, the HP-β-CD is supplied by various providers with different MS, mainly as MS 0.65 and 0.85, which are prevalently used in pharmaceutical and cosmetic field, respectively.

Considering this, the first purpose of this work was to improve aqueous solubility of MXD using the inclusion complex strategy avoiding organic solvents. In particular, 2 pharmaceutical grade HP-β-CD having different MS were studied and compared to select the one with better solubilizing properties which allowed us to formulate and characterize new alginate-based hydrogels containing concentration of MXD in the range of 1%-6% w/w.

Material and Method

Material

MXD (2,4-diamino-6-piperidinopyrimidine 3-oxide, MW 209.25, 99% purity), HP-β-CD (MS 0.65) MW average 1396 and sodium alginate (viscosity 1% at 25°C 500-600 mPa s) were gifted by Farmalabor srl (Canosa di Puglia, Italy). HP-β-CD (MS 0.85), MW average 1480, was given by Roquette (Cassano Spinola, Italy). All other reagents used in this work were of analytical grade or high performance liquid chromatography (HPLC) grade, and double-distilled water was used.

Methods

Determination of MXD by HPLC

A previous validated method was used to quantify the MXD by HPLC with some modification.[10] Briefly, 20-μL sample was injected into a C18 column (Zorbax SB Aq 15 cm × 4.6 mm; 4 μm particles; Agilent) using a manual loop (Reodyne). The column was eluted with an isocratic mobile phase consisting of methanol and water (75/25, v/v) at a flow rate of 0.8 mL/min with a Waters model 1515 pump (Waters Corp., Milford, MA). Detection was performed by UV-Vis spectrophotometer (Waters 2487). Standard calibration curves were obtained at 285 nm using CH_3OH/H_2O (75/25) as solvent and were linear ($R^2 > 0.999$) over the range of tested concentrations (0.03-9.37 × 10^{-5} mg/mL). The retention time of MXD was 2.0 min.

Preparation and Characterization of MXD/HP-β-CD Inclusion Complex in Solution

Phase Solubility Studies. Solubility studies were performed according to the method reported by Higuchi and Connors.[22] Phase solubility studies with HP-β-CD MS 0.65 or 0.85 were performed in water at 25 ± 0.5°C using screw cap tubes. In more detail, an amount of MXD (0.40 M) greater than the intrinsic aqueous solubility was added to 4.0 mL of water with increasing amount of HP-β-CD until 0.23 M. Each suspension was kept in a shaking water bath for 24 h at 25 ± 0.5°C and then filtered through 0.22-μm cellulose acetate membrane filter. The filtrated samples were analyzed by HPLC after appropriated dilution with a mixture of CH_3OH/H_2O (75/25, v/v). The unknown concentration of MXD in each analyzed solution was determined using a calibration curve. Earlier experiments showed that the presence of HP-β-CD did not interfere with the assay at the employed concentration. The phase solubility profile was obtained by plotting the molar concentration of MXD (M) versus the molar concentration of HP-β-CD (M). Complexation constants (K_c) were calculated from phase solubility plot, and the results were the average of 3 different experiments.

Thermodynamic Parameters for the Inclusion Reaction of MXD With HP-β-CD. The thermodynamic parameters (ΔH^0, ΔS^0, and ΔG^0) for the formation of inclusion complex MXD/HP-β-CD were determined from temperature dependence of K_c, by using classical van't Hoff's equation (Eq. 1), plotting ln K_c versus 1/T.[23]

$$\ln K_c = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (1)$$

where R is universal gas constant (J mol^{-1} K^{-1}); T is the absolute temperature (Kelvin), and ΔH^0 (J mol^{-1}) and ΔS^0 (J mol^{-1} K^{-1}) are variation on enthalpy and entropy, respectively.

The K_c at the temperature of 37°C and 45°C were calculated by phase solubility studies as reported above at 25°C.

Proton Nuclear Magnetic Resonance Spectroscopic Studies. Proton nuclear magnetic resonance (^{1}H-NMR) spectra, and 2D correlations spectroscopy (COSY), nuclear overhauser effect spectroscopy (NOESY), and rotating-frame overhauser enhancement spectroscopy (ROESY) experiments were recorded at 25°C in D_2O, with an Agilent (VNMRS500) 500-MHz spectrometer. The values of the coupling