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Preparation and characterisation of hexamidine salts

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ABSTRACT

Hexamidine diisethionate (HEX D) has been used in the personal care industry and in a number of over-the-counter (OTC) drug products as an antimicrobial agent since the 1950's. Recently, the compound has also been investigated for its beneficial effects on skin health. Surprisingly, there is only limited information describing the physicochemical properties of this compound in the literature. The objective of this work was therefore to conduct a comprehensive programme of characterisation of HEX D as well as its dihydrochloride salt (HEX H). HEX H was prepared from HEX D by a simple acid addition reaction. Both salts were characterised using Nuclear Magnetic Resonance (NMR), Differential scanning calorimetry (DSC), and Thermogravimetric analysis (TGA). A new high performance liquid chromatographic method was developed and validated for both compounds. The pH in aqueous solution as well as respective distribution coefficients between octanol and pH 7.4 buffer were also determined. Finally, solubility and short term stability studies were conducted in a range of solvents. NMR analysis confirmed the preparation of HEX H from HEX D. Thermal analysis indicated the melting points of HEX D and HEX H were 225 °C and 266 °C respectively. HPLC analysis confirmed the purity of both salts. Log D values at pH 7.4 were −0.74 for HEX D and −0.70 for HEX H respectively. The physicochemical properties of two HEX salts have been established using a range of analytical approaches. Detailed solubility and stability data have also been collated. This information will be useful in the design of novel formulations for targeted delivery of these compounds to the skin.

1. Introduction

Hexamidine (HEX) is an aromatic diamidine and a strong organic base. Although primarily used as the diisethionate salt (HEX D), it was firstly synthesised as the dihydrochloride (HEX H) and patented by Ewins et al. (1939) for May & Baker Limited (U.K.). The company was interested in the trypanocidal activity of the diamidines and the dihydrate of HEX H was subsequently demonstrated to be the most potent of the group (Ashley et al., 1942). Antiprotozoal activity was demonstrated more than 50 years later when Brasseur et al. (1994) used HEX D to treat two subjects affected by *Acanthamoeba* keratitis. HEX D has also shown efficacy against *Pseudomonas aeruginosa*, *Proteus*, *Escherichia coli*, *Staphylococcus aureus* and *Tsukamurella paurometabolum* (van Ketel, 1975; Granel et al., 1996). A more recent *in-vitro* study demonstrated HEX D efficacy against a series of multi-drug resistant gram-positive bacteria (Grare et al., 2010). Geratz et al. (1973) demonstrated the efficacy of HEX H dihydrate as an enzyme inhibitor with K_i values of 1.9, 4.5 and 7.4 μM, trypsin, pancreatic kallikrein and thrombin respectively. Enyedy et al. (2001) confirmed HEX inhibitory activity against thrombin (K_i value 224 nM) and matriptase (K_i = 924 nM), but did not specify if the active was used as the free base or salt. Finally, an *in-vivo* study investigated the effect of two HEX salts on nitric oxide synthase (NOS). Surprisingly, while HEX D significantly decreased NOS activity, the tetrachloroplatinate (II) salt had no effect on NO generation (Morgant et al., 1998).

A number of publications have focussed on the role of HEX as an anti-aging and moisturising active in cosmetics and specifically the influence of HEX on various biomarkers of corneocyte maturity and skin turnover. Kimball et al. (2012) speculated that HEX might attenuate the skin ageing process because of its inhibitory activity on serine proteases associated with skin inflammation. Both skin inflammation and abnormal lipid biosynthesis have been linked to skin ageing (McGrath et al., 2012). Osborne et al. (2009) and Jarrold et al. (2010a) showed that when human skin equivalent cultures were exposed to HEX, cholesterol, fatty acid and sphingolipid biosynthesis as well as cholesterol and fatty acid uptake were downregulated while cholesterol efflux was upregulated. Jarrold et al. (2010b) demonstrated that the application of a cosmetic moisturiser containing HEX, niacinamide and palmitoyl-lysine-

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threonine significantly increased the number and size of mature corneocytes of the facial stratum corneum of twenty female subjects. Significant thickening of the stratum corneum (SC) as well as a reduction in transepidermal water loss of the volar forearm was reported for 36 female subjects following treatment with a cream containing HEX and niacinamide (Kaczvinsky et al., 2010). However these *in vivo* studies did not specify if the active was used as the free base or salt.

The safety of HEX and HEX D has been assessed by the Cosmetic Ingredient Review Expert Panel (2007). The panel concluded that both actives are safe when used in cosmetics at concentrations less than or equal to 0.10%. This opinion was subsequently confirmed by the European Parliament and the Council of European Union (2009) which fixed the maximum allowed concentration of HEX and its salts in cosmetic products to 0.10%. However, several cases of allergic contact dermatitis have been reported since HEX has been in use (Gougerot et al.1950; Sidi et al., 1969; van Ketel, 1975; Robin,1978; Dooms-Goossens et al., 1989; Brand and Ballmer-Weber, 1995; Mullins, 2006).

To date, HEX D has been used as a preservative in ~40 cosmetic products and in a number of over-the-counter formulations (Cosmetic Ingredient Review Expert Panel, 2007). Surprisingly, there is only a limited amount of information describing the physicochemical properties of HEX in the literature (British Pharmacopoeia Commission, 2014). The use of HEX H as an alternative salt to HEX D has also not been explored. The objective, therefore, of the present work, was to undertake a comprehensive programme of characterisation of HEX D and HEX H (Fig. 1). In the longer term this information should assist in the design of formulations which target this active more effectively to the skin.

2. Materials and methods

2.1. Materials

HEX D (Laboratoires Sérobiologiques, France) was a gift from Procter and Gamble (U.S.A.), while HEX H was synthesized and purified in-house. Propylene glycol, polyethylene glycol 200, HPLC grade isopropyl alcohol, trifluoroacetic acid (HPLC grade) and absolute ethanol were supplied by Fisher Scientific (U.K.). HPLC grade solvents (acetonitrile, methanol, water), glycerol, isopropyl myristate, 1-octanol, 2-ethylhexyl salicylate, 1 M hydrochloric acid solution and dimethyl sulfoxide-d_6 were provided by Sigma–Aldrich (U.K.). Dimethyl sulfoxide was supplied by VWR International (U.K.). Propylene glycol monolaurate, Labrafac™ PG and Transcutol® P were received as gifts from Gattefossé (France). 1,2-pentanediol was provided by Surfachem Group (U.K.). Dimethyl isosorbide (Arlasolve®) was supplied by Croda International (U.K.). Oleic acid was provided by Fluka (U.K.). Miglyol® 812 N was supplied by Sasol (Germany). Dipropylene glycol was provided by Acros Organics (Belgium). Phosphate buffered saline was prepared using Dulbecco A tablets (Oxoid, U.K.).

2.2. Methods

2.2.1. Conversion of HEX D to HEX H

Approximately 50 mL of 1 M hydrochloric acid solution were heated at 100 ± 1 °C using an Ikamag® C-MAG HS 7 magnetic stirrer ceramic heating plate (IKA, Germany) equipped with an ETS-D5 electronic contact thermometer (IKA, Germany). HEX D was dissolved in the solution followed by stirring of the mixture and cooling (15 min). The flask was subsequently placed on ice for 30 min to allow recrystallisation of the product. Finally, crystals were recovered by means of vacuum filtration and dried at room temperature. Hydrogen-1 and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy were used to confirm the structure of the starting material and the product of the reaction. All spectra were acquired in dimethyl sulfoxide-d_6 on a Bruker Avance 400 MHz NMR spectrometer (Bruker Corporation, U.S.A.) and processed using MestReNova® 9.0.1 (Mestrelab Research, Spain).

2.2.2. Thermal analysis

The melting points of HEX D and HEX H were examined using thermogravimetric analysis (TGA) and differential scanning

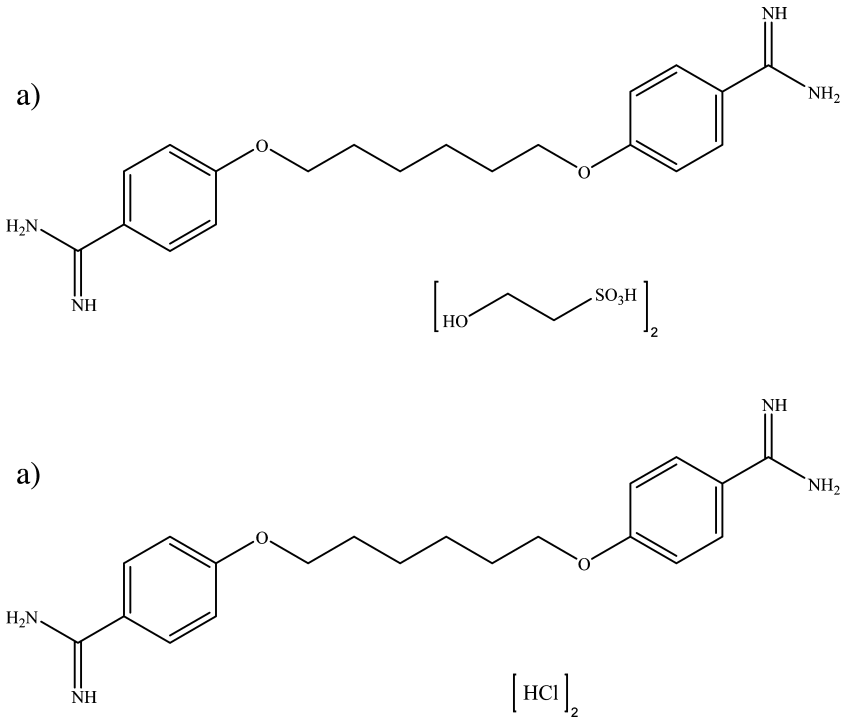

Fig. 1. Chemical structures of (a) HEX D and (b) HEX H.