International Journal of Cosmetic Science

Hexamidine salts – applications in skin health and personal care products

Journal:	*International Journal of Cosmetic Science*
Manuscript ID	Draft
Manuscript Type:	Review Article
Keywords:	Hexamidine, salt, biocide, protease inhibitor

Abstract

Hexamidine (HEX) has been used as a preservative in topical preparations since the 1950s. A number of studies also indicate that the molecule plays a beneficial role in skin homeostasis. In this review we describe the physicochemical properties of hexamidine diisethionate (HEX D) and the corresponding hydrochloride salt (HEX H). The biocidal and protease inhibition properties of HEX are outlined as well as the effects of HEX on lipid processing enzymes, corneocyte maturity, stratum corneum thickness and Trans epidermal water loss (TEWL). Skin permeation properties of HEX D and HEX H are summarised and formulation approaches for effective dermal targeting of HEX are discussed.

Key words: Hexamidine, salt, biocide, protease inhibitor, skin, formulation

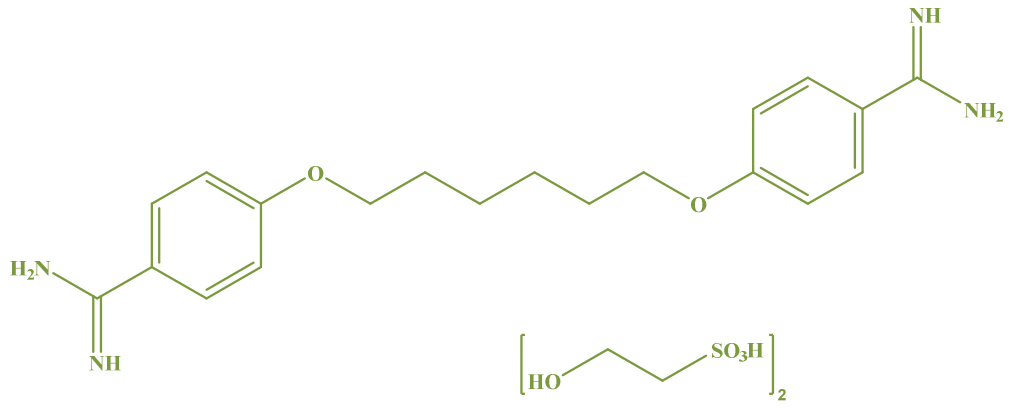
Introduction

Hexamidine (HEX) is a strong organic base and is an aromatic diamidine. Although hexamidine is primarily used as the diisethionate salt (HEX D), it was firstly synthesised as the dihydrochloride salt (HEX H) and patented for May & Baker Limited (U.K.), by Ewins *et al.* [1] (1939). The company was interested in the promising trypanocidal activity of the aromatic diamidines and subsequently demonstrated that hexamidine dihydrochloride dihydrate (HEX H) was the most potent of all compounds synthesised [2] (Ashley *et al.*, 1942). In the 1990's the efficacy of HEX as an amoebicidal agent was demonstrated in a number of studies [3,4] (Brasseur et al., 1994; Perrine et al., 1995; Gray et al., 1996). In addition to biocidal activity HEX and other diamidines have demonstrated enzyme inhibition properties [5] (Geratz et al., 1973). Upregulation of the major cholesterol and fatty acid uptake pathways has also been demonstrated in a skin equivalent tissue culture model following treatment with HEX [6] (Jarrold et al., 2010b). Surprisingly, until relatively recently very little information about the physicochemical properties and dermal disposition of HEX had been reported. The aims of this review are to (i) Summarise the physicochemical properties of hexamidine and its salts (ii) Outline the applications of HEX as a biocide (iii) Critically assess the studies which have investigated the potential benefits of HEX for skin health (iv) Identify appropriate dermatological vehicles for effective delivery of HEX to the skin.

Physicochemical properties and analytical methods to quantify hexamidine diisethionate (HEX D) and hexamidine dihydrochloride (HEX H)

Hexamidine diisethionate

The salt of hexamidine with the strong organic isethionic acid, a member of the sulphonic acid series, is the only hexamidine derivative currently reported in the British Pharmacopoeia [7] (British Pharmacopoeia, 2014). The chemical structure, IUPAC name, chemical formula and molecular weight of hexamidine diisethionate (HEX D) are summarised in Figure 1.



IUPAC Name: 4,4'-[hexane-1,6-diylbis(oxy)]dibenzimidamide bis(2-hydroxyethane-1-sulfonate)

Chemical Formula: $C_{24}H_{38}N_4O_{10}S_2$

Molecular Weight: 606.71 g/mol

Figure 1 Chemical structure, IUPAC name and chemical formula of HEX D

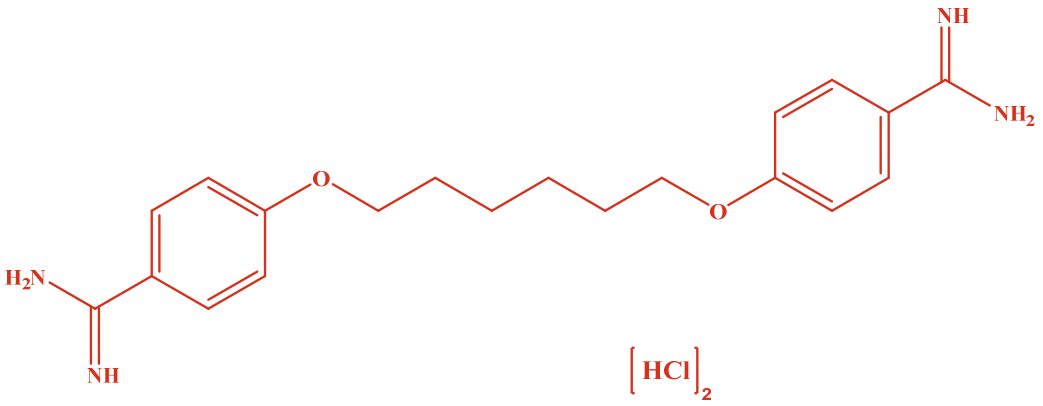
HEX D is described in the British Pharmacopoeia 2015 monograph as a "white or slightly yellow powder, hygroscopic, sparingly soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in methylene chloride" [7] (British Pharmacopoeia Commission, 2014). Recently we reported the characterisation of HEX D [8] (Parisi et al., 2015) and described a number of physicochemical properties which have not been reported previously including solubility, melting point and Log D at pH 7.4 (Table 1). We also developed a new High Performance Liquid Chromatographic method for analysis of HEX D. Two other HPLC methods are described in the literature. The first was developed in order to quantify HEX D in various pharmaceutical formulations such as creams, ointments and eyewash solutions [9] (Taylor *et al.*, 1983). The purpose of the second was the qualitative separation of HEX D from other actives with similar chemical structure and its quantification in a cosmetic cream [10] (De Bukanski and Masse, 1984). Since HEX D has typically been used in solution no variations in its crystal structure (polymorphism) had been reported until recently. Fucke *et al.* (2008) Fucke et al. [11] used X-ray crystallisation and thermal analysis to identify ten anhydrous crystal forms and two dihydrates of HEX D.

Table 1. Physicochemical properties of HEX D and HEX H [8] (Parisi et al., 2015)

	HEX D	**HEX H**
Molecular weight	606.7	427.4
Melting point (°C)	224.9	265.5
Solubility (mg/mL), 32°C	52.0	16.0
Log $D_{(o/w)}$ pH 7.4	− 0.74 ± 0.02	− 0.70 ± 0.02
pH in aqueous solution	6.3 – 6.4	6.3 – 6.4

Hexamidine dihydrochloride

As noted, hexamidine was first synthesised as the dihydrochloride salt in the late 1930's but, subsequently, the diisethionate salt was preferred for use, presumably because of its more favourable water solubility. The preparation and characterisation of HEX H (Figure 2) was recently reported [8] (Parisi et al., 2015) and the physicochemical properties of the salt are summarised in Table 1. A new HPLC method was also developed for analysis of HEX H. With reference to topical delivery, HEX H has more favourable properties than HEX D, with a lower molecular weight and higher Log D value (pH 7.4). Consistent with these values, we have recently demonstrated >70% delivery of HEX H to porcine skin, *in vitro*, using a simple binary mixture of propylene glycol and propylene glycol monolaurate [12] (Parisi et al., 2016). In contrast, the maximum amounts of HEX D that could be delivered were of the order of 30%.



IUPAC Name: 4,4'-[hexane-1,6-diylbis(oxy)]dibenzimidamide dihydrochloride

Chemical Formula: $C_{20}H_{28}Cl_2N_4O_2$

Molecular Weight: 427.37 g/mol

Figure 2. Chemical structure, IUPAC name and chemical formula of HEX H

Applications and uses of hexamidine as a biocide

As noted earlier, hexamidine was initially developed as a trypanocidal agent [1] (Ewins et al., 1939). The antiprotozoal activity of hexamidine was further explored more than 50 years later when Brasseur *et al.* (1994) Brasseur et al. [3] successfully used HEX D to treat two subjects affected by *Acanthamoeba* keratitis. In addition, an *in-vitro* study from Perrine *et al.* (1995) Perrine et al. [4] showed that HEX D was effective not only against *Acanthamoeba* trophozoites but also against the dormant cyst forms. Bailly *et al.* (1997) Bailly et al. [13] thus hypothesised that the amoebicidal activity of hexamidine might have been directly related to its capacity to selectively bind DNA. However, although the study showed that HEX D strongly bound DNA, no correlation was found between the amoebicidal potency of the aromatic diamidines series and their DNA binding ability. In contrast, HEX D failed when used against an *Acanthamoeba* and *Hartmannella* corneal coinfection [14] (Aimard *et al.*, 1998) and showed only limited efficacy on six subjects affected by chronic *Acanthamoeba* keratitis [15] (Pérez-Santonja *et al.*, 2003).

Interestingly, HEX H 2.5-hydrate was also found to be active against pneumonia induced in a rat model by a yeast-like fungus as *Pneumocystis carinii* [16] (Tidwell et al., 1990). As observed earlier, for *Acanthamoeba*, DNA binding was proposed as a possible mechanism of action, but no direct correlation was observed between anti *Pneumocystis carinii* activity and DNA binding.

In terms of antibacterial properties, HEX D has been reported to be effective against *Pseudomonas aeruginosa*, *Proteus*, *Escherichia coli*, *Staphylococcus aureus* and *Tsukamurella paurometabolum* [17,18] (van Ketel, 1975; Granel *et al.*, 1996). In addition, a more recent *in-vitro* study demonstrated the efficacy of HEX D against a series of multi-drug resistant gram-positive bacteria [19] (Grare *et al.*, 2010).

The exact mechanism of diamidine antibacterial efficacy, including HEX D, is still unclear. However, due to its native positive charge, it is thought that HEX D binds with high affinity to the negatively-charged cell walls and membranes of bacteria and that disruption is brought about by perturbations of these binding sites [20] (Kratzer et al., 2006), resulting in inhibition of oxygen uptake and induced leakage of amino acids. In this sense, HEX D might be considered to be acting as a cationic surface-active agent [21] (McDonnell & Russell, 1999).

Contrasting antibacterial efficacy was reported by Murray et al. [22] Murray *et al.* (2004) who claimed that HEX D was both an inducer and substrate of the *Staphylococcus aureus* multidrug efflux transporter. Furthermore, Lemaître et al. [23] Lemaître *et al.* (1998) identified 14 strains of *Listeria* resistant to HEX D. The authors attributed this resistance to plasmid-mediated acquisition of a *Staphylococcus aureus* replicon.

The impact of HEX D on human cutaneous microbiota has been investigated by Michel et al. [24] Michel *et al.* (1986) and Chevalier and Cremieux [25] Chevalier and Crémieux (1992). Both research groups concluded that HEX D is an effective and fast-acting antiseptic. However, while Michel et al. [24] Michel *et al.* (1986) reported a marked disturbance of the skin microbiota, only slight modifications of the bacterial population were observed by Chevalier and Cremieux [25] Chevalier and Crémieux (1992).

In line with the broad spectrum of antimicrobial activity shown by hexamidine in its various forms, the European Union Cosmetics Directive 76/768/EEC, Annex VI, allows HEX D as a preservative for cosmetics and toiletries up to a maximum concentration of 0.10% [26] (European Economic Community 2001). Furthermore, in a submission by the US Cosmetics, Toiletries and Fragrances Association to the FDA in 2002, HEX D was listed as a preservative in 38 cosmetic products [27-29] (FDA, 2002; CTFA 2004;Cosmetic Ingredient Review Expert Panel, 2007).

Hexamidine as an enzyme inhibitor

The aromatic diamidines series has also been studied to determine any enzyme inhibition properties. Geratz et al. [5] Geratz *et al.* (1973) examined their ability to inhibit trypsin, pancreatic kallikrein and thrombin; HEX H dihydrate was effective against all enzymes with reported K_i values of 1.9, 4.5 and 7.4 μM, respectively. Enyedy et al. [30] Enyedy *et al.* (2001) confirmed hexamidine inhibitory activity against thrombin although a considerably lower K_i value (224 nM) was reported; however these authors did not specify if the active was used as the free base or salt form. Furthermore, the study demonstrated that hexamidine was able to inhibit matriptase (K_i = 924 nM), a trypsin-like serine protease involved in tissue remodelling, cancer invasion and metastasis. Finally, an *in-vivo* study performed by Morgant et al. [31] Morgant *et al.* (1998) investigated the effect of two hexamidine salts on nitric oxide synthase (NOS). Surprisingly, while the diisethionate salt significantly decreased NOS activity, the tetrachloroplatinate (II) salt had no effect on NO generation.

Hexamidine and skin biology

Kimball *et al.* (2012) Kimball et al. [32] speculated that hexamidine 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 [33] (McGrath *et al.*, 2012). Osborne et al. [34] and Jarrold et al. [6] Osborne *et al.* (2009) and Jarrold *et al.* (2010b) showed that the application of hexamidine in human skin equivalent cultures was able to reverse a series of cellular processes which are typical of aged skin. These processes included the downregulation of cholesterol, fatty acid and sphingolipid biosynthesis, the downregulation of cholesterol and fatty acid uptake and the upregulation of cholesterol efflux. Other studies have highlighted the ability of hexamidine to enhance the barrier properties of stratum corneum. Jarrold et al. [35] Jarrold *et al.* (2010a), for example, demonstrated that the application of a cosmetic moisturiser containing hexamidine, niacinamide and Pal-KT significantly increased the number and size of mature corneocytes of the facial stratum corneum of twenty female subjects. A further study showed a significant thickening and a concurrent reduction in transepidermal water loss (TEWL) for the volar forearm in 36 female subjects, following treatment with a cream containing hexamidine and niacinamide [36] (Kaczvinsky *et al.*, 2010).

Safety of hexamidine with reference to skin application

The European Union Cosmetics Directive 76/768/EEC, Annex VI, allows HEX D as a preservative for cosmetics and toiletries up to a maximum concentration of 0.10% [26] (European Economic Community 2001). The safety of hexamidine and HEX D was assessed by the Cosmetic Ingredient Review Expert Panel [29] Cosmetic Ingredient Review Expert Panel (2007), which 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 [37] European Parliament and the Council of European Union (2009) which fixed the maximum allowed concentration of hexamidine and its salts in cosmetic products at 0.10%. Nevertheless, several cases of allergic contact dermatitis have been reported with hexamidine and the first was reported by Gougerot et al. [38] Gougerot *et al.* (1950). Sidi et al. [39] Sidi *et al.* (1969) observed 147 cases of sensitization to HEX D in 8 years. Further cases were described by van Ketel [17], Robin [40], Dooms-Goosens et al. [41] and Brand and Ballmer-Weber [42]. van Ketel (1975), Robin (1978), Dooms-Goossens *et al.* (1989) and Brand and Ballmer-Weber (1995). One case of particular interest was reported by Mullins [43] Mullins (2006) who observed an allergic systemic reaction due to topical application of HEX D. Furthermore, in a study aimed at comparing 75 cases of contact dermatitis caused by antiseptics, hexamidine was the strongest sensitizer with 20 positive patch tests [44] (Barbaud *et al.*, 2005). However, studies which involve larger numbers of subjects have shown that sensitization to hexamidine is not a common phenomenon. For example, Roul et al. [45] Roul *et al.* (1999) tested 269 children aged 3 to 15 years with 34 allergens and attributed only 1 allergic reaction to hexamidine. In a larger study, 641 children less than 16 years of age with atopic dermatitis were patch tested with 7 actives which are commonly used for the topical treatment of this disease [46] (Mailhol *et al.*, 2009). The results showed that HEX D caused allergic contact dermatitis to only 3 children (0.5% of tested population). It can thus be concluded that hexamidine and its salts are generally safe to use, but adverse reactions are possible because of their skin-sensitizing properties.

Topical delivery of hexamidine salts to the skin

Dermal absorption of HEX in human skin has not been studied extensively but a number of studies have been conducted in other models for toxicity and irritation evaluation. *In vitro* penetration of HEX from gel (0.1%) or cream (0.1, 0.3%) formulations was investigated in human cadaver skin using Franz cells [29] (Cosmetic Ingredient Review Expert Panel, 2007). Following finite dose application, 0.027% of HEX in the gel

formulation penetrated the skin samples in 72 h. In the 0.1% and 0.3% water-oil formulations, 0.0321% and 0.0208% of the HEX dose, respectively, penetrated the skin samples in 72 h. The authors suggested the low skin penetration observed in this study indicated that bioavailability of HEX from topical cosmetic formulations would be minimal

Recently we outlined a rational formulation approach for effective targeting of HEX D and HEX H to the skin [12] (Parisi et al., 2016). Based on the solubility data reported in our earlier study [8] (Parisi et al., 2015), propylene glycol (PG), glycerol and PEG 200 were confirmed as suitable solvents. In addition to individual solvents, binary systems which combined the solvents with other chemical penetration enhancers (CPEs) were prepared and evaluated. CPEs were selected based on compatibility with PG, glycerol and PEG 200 and their reported applications in dermal formulations [47] (Lane, 2013). Candidate vehicles were evaluated using porcine skin mounted in *in vitro* Franz cells. The solvents and CPEs examined were dimethyl isosorbide (DMI), glycerol, isopropyl alcohol (IPA), 1,2-pentanol (1,2-PENT), polyethylene glycol (PEG) 200, propylene glycol (PG), propylene glycol monolaurate (PGML) and Transcutol®P (TC). Formulations containing PGML were particularly efficacious compared with other solvents or binary combinations. A binary system of PG:PGML (50:50) delivered ~70% of HEX H to the skin and also promoted skin penetration of HEX D (30%).

Conclusions

Although HEX was originally synthesised as the dihydrochloride salt, today it is the diisethionate form which is used in personal care products. The comprehensive characterisation of the physicochemical properties of both salts should facilitate the future development of novel formulations for topical delivery of HEX. Despite the fact that HEX H has more suitable properties for skin permeation than HEX D, *in vitro* permeation data indicate no advantage of the dihydrochloride salt over the diisethionate for dermal delivery. To date, HEX has been shown to affect biomarkers of skin barrier function, such as corneocyte size and maturity, lipid biosynthesis and uptake, increase stratum corneum thickness, reduce TEWL and modulate the activity of serine proteases associated with inflammation and turnover of the skin. However, further studies at the molecular level are needed to probe the interaction of HEX with cellular processes. HEX is incorporated in extremely low amounts in consumer products and it would also be interesting to examine any dose response effects *in vitro* and/or *in vivo*.

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