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N-Chlorotaurine and ammonium chloride: An antiseptic preparation with strong bactericidal activity

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Abstract

The bactericidal activity of the endogenous antiseptic *N*-chlorotaurine (NCT) is significantly enhanced in the presence of ammonium chloride which induces the formation of monochloramine (NH_2Cl) whose strong bactericidal activity is well known. In this study the properties of NCT plus ammonium chloride have been investigated. The reaction of active chlorine compounds like chloramine-T (*N*-chlorotoluenesulfonamide sodium), chloroisocyanuric acid derivatives, hypochlorites (NaOCl, $CaOCl_2$) with ammonium chloride did not stop at the stage of monochloramine, and the pungent smelling by-products di- and trichloramine, $NHCl_2$ and NCl_3, were also formed. This was not the case with NCT where only monochloramine was generated. The equilibrium constant of the reaction of NCT with ammonium was found to be $K_{NCT/NH_4} = [NH_2Cl][Tau]/[NCT]/[NH_4^+]/f_a^2 = (5.8 \pm 1.2)E{-}3$, which allows to estimate the equilibrium concentration of monochloramine in aqueous solutions of NCT and ammonium chloride. At concentrations each ranging between 0.01% and 1.0% it comes to $[NH_2Cl] = 3.5$–254 ppm. As an unexpected result the monochloramine containing formulation turned out to be most stable in plain water without buffer additives. Quantitative killing assays revealed complete inactivation of 10^6 to 10^7 CFU/mL of seven bacterial strains by 0.1% NCT plus 0.1% ammonium chloride within 5 min, while with plain 0.1% NCT an incubation time of 2–4 h was needed to achieve the same effect. The highly significant increase of bactericidal activity (200–300-fold) could be assigned to the presence of monochloramine which could be isolated by vacuum distillation. Aqueous solutions of NCT and ammonium chloride provide a highly effective and well tolerable antiseptic preparation appropriate to a treatment cycle of at least 1 month if stored in the refrigerator.

Keywords: Monochloramine; Microbicidal activity; Antiseptic; Topical application

1. Introduction

The bactericidal activity of NCT[1] in body fluids resulted, despite of an assessed consumption of oxidation capacity, in an increased activity compared to plain buffer solutions (Nagl and Gottardi, 1996; Nagl et al., 2001). As a reason transhalogenation equilibria with components bearing N–H bonds have been realized which cause the formation of their *N*-chloro derivatives:

$$R\text{–}NH_2 + NCT + H^+ \leftrightarrow R\text{–}NHCl + \text{taurine}$$

It was found that the *N*-chloro derivatives of free amino-carbonic acids (compared to the amino-sulfonic acid taurine) and above all ammonia, i.e. NH_2Cl, are responsible for this beneficial effect (Nagl and Gottardi, 1996; Nagl et al., 2001). Most impressively, a mixture of NCT and ammonium chloride showed fast killing of important pathogens like mycobacteria (Nagl and Gottardi, 1998) and fungi (Nagl et al., 2001). The prominent bactericidal qualities of the formed monochloramine were reduced to its small dimension and the lack of an electric charge, properties which facilitate penetration into the bacterial cell (Thomas, 1979; Grisham et al., 1984).

However, the superior performance of NH_2Cl is curtailed by instability, a flaw which applies not to NCT. For the instabil-

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[1] Because *N*-chlorotaurine and *N*,*N*-dichlorotaurine are strong acids, their salts are completely dissociated. The abbreviations NCT and NDCT, therefore, concern the anions $ClHN\text{–}CH_2\text{–}CH_2\text{–}SO_3^-$ and $Cl_2N\text{–}CH_2\text{–}CH_2\text{–}SO_3^-$, respectively, which are responsible for the reactions quoted in this paper. The solid alkali salts are specified by NCT-Na and NDCT-Na.

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ity of NH_2Cl auto-decomposition models have been established (Vikesland et al., 2001) including the disproportionations Eqs. (1) and (2) and the redox-reaction Eq. (3):

$$2NH_2Cl \leftrightarrow NH_3 + NHCl_2 \quad (1)$$

$$NHCl_2 + NH_2Cl \leftrightarrow NCl_3 + NH_3 \quad (2)$$

$$NHCl_2 + NH_2Cl \rightarrow N_2 + 3H^+ + 3Cl^- \quad (3)$$

Notwithstanding its instability, NH_2Cl became important in water disinfection (Daniel et al., 1993) where it is produced *in situ* from ammonia or ammonium salts and elemental chlorine or active chlorine compounds (bearing O–Cl or N–Cl functions).

These results and the beneficial properties of plain NCT as an antiseptic in human medicine (Koprowski and Marcinkiewicz, 2002; Nagl et al., 2003; Neher et al., 2004, 2005; Romanowski et al., 2006; Teuchner et al., 2005) arose the question if NCT could be used as a chlorine source for the production of NH_2Cl or, with other words, if the combination of NCT and ammonium chloride[2] is suited as an antiseptic preparation in human medicine. The aim of this study, therefore, was to elaborate the adequate conditions to provide *in situ* defined concentrations of NH_2Cl for practical use. To gain these ends, besides continuative killing tests, also a thorough investigation of the reaction of NCT with ammonium (Eq. (4)) including a re-determination of its equilibrium constant (Gottardi and Nagl, 2002) was required.

2. Materials and methods

2.1. Chemical experiments

2.1.1. Chemicals

Ammonium chloride, chloramine-T and buffers were from Merck (Darmstadt, Germany), dichloro isocyanuric acid from Fluka (Buchs, Switzerland). *N*-Chlorotaurine sodium (NCT-Na) and *N*,*N*-dichlorotaurine sodium (NDCT-Na) were synthesized after (Gottardi and Nagl, 2002; Gottardi et al., 2005). All reagents were of the highest available purity. The concentrations in percent were weight per volume (w/v).

2.1.2. Assessment of oxidation capacity

Iodometric titrations were performed with 0.100 M thiosulfate at pH 2–3 (acetic acid) using the automatic titration assembly TIM900 from Radiometer, Copenhagen.

2.1.3. Photometric measurements

The DU-800 spectrophotometer from Beckman-Coulter was used with a preset resolution of 0.1 nm. The absorption coefficients were based on the oxidation capacity (c(Ox)) of solutions of the pure compounds assessed by iodometric titration (see above). Measurements were conducted at 25 °C.

[2] Principally every non-toxic ammonium salt could be used for NH_2Cl production. However, because of the ubiquitous presence of chloride in the human body ammonium chloride was considered as most qualified.

2.1.4. Algorithm for the simultaneous analysis of NH_2Cl, NCT and NDCT

For the absorptions A_1, A_2, and A_3 of a system with three components applies

$$A_1 = aX + bY + cZ; \qquad A_2 = dX + eY + fZ;$$

$$A_3 = gX + hY + jZ$$

By substitution follows

$$Z = \frac{(gb - ah)(gA_2 - dA_3) + (ge - dh)(aA_3 - gA_1)}{(gf - dj)(gb - ah) - (gc - aj)(ge - dh)}$$

$$Y = \frac{gA_2 - dA_3 - Z(gf - dj)}{ge - dh}; \qquad X = \frac{A_3 - hY - jZ}{g}$$

where X, Y and Z are the molar concentrations of NH_2Cl, NCT, and NDCT. As analytical wavelengths were chosen 237.0 nm (A_1), 270.5 nm (A_2), and 302.6 nm (A_3) which gave sufficient differences concerning absorptivity.

The molar absorption coefficients of NH_2Cl, NCT, and NDCT are specified by: a, b, and c at 237.0 nm, with $a = 417.9\,L\,mol^{-1}\,cm^{-1}$, $b = 318.9\,L\,mol^{-1}\,cm^{-1}$, and $c = 675.2\,L\,mol^{-1}\,cm^{-1}$; d, e, and f at 270.5 nm, with $d = 139.1\,L\,mol^{-1}\,cm^{-1}$, $e = 260.5\,L\,mol^{-1}\,cm^{-1}$, and $f = 102.8\,L\,mol^{-1}\,cm^{-1}$; g, h, and j at 302.6 nm, with $g = 4.42\,L\,mol^{-1}\,cm^{-1}$, $h = 24.7\,L\,mol^{-1}\,cm^{-1}$, and $j = 328.1\,L\,mol^{-1}\,cm^{-1}$.

Annotations.

(1) The theoretical error of the method primarily can be reduced to the accuracy of the three absorptions and the nine absorption coefficients which can be estimated to $A \pm 0.001$ and $\varepsilon \pm 1\%$, respectively.

Using these spans an error of 2.5% can be assigned to [NH_2Cl].

The experimental S.D. of repeated measurements each with preceding blanking yielded an error of only 0.3% (coefficient of variation).

(2) The results of the UV-method were verified by iodometric titration at pH 2–3 (gives the oxidation capacity c(Ox) = [NH_2Cl] + [NCT] + 2[NDCT]) and resulted in a good congruence which, however, decreased with the storing period. A similar observation was made by Valentine et al. (1986) with chloraminated drinking water which they attributed to unidentified decomposition products.

(3) Because of the only minor spectral differences of NCT and NH_2Cl (λ_{max} = 251 resp. 244 nm) the results are reliable only if both compounds are present in comparable concentrations.

(4) An alternative for estimating the NH_2Cl concentration consists in assessing the NH_2Cl capacity by vacuum distillation (see below).

2.1.5. Determination of the equilibrium constant K_{NCT/NH_4}

The mass-law expression for the reaction

$$NCT + NH_4^+ \leftrightarrow NH_2Cl + Tau \quad (4)$$