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

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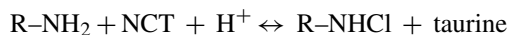
## Abstract

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*-chlorotoluene-sulfonamide 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.
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*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:
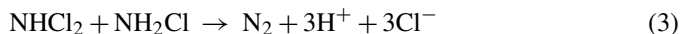
$$R–NH_2 + NCT + H^+ \leftrightarrow R–NHCl + \text{taurine}$$

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 $ClHN–CH_2–CH_2–SO_3^-$ and $Cl_2N–CH_2–CH_2–SO_3^-$, respectively, which are responsible for the reactions quoted in this paper. The solid alkali salts are specified by NCT-Na and NDCT-Na.

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):

$$2NH_2Cl \leftrightarrow NH_3 + NHCl_2 \tag{1}$$

$$NHCl_2 + NH_2Cl \leftrightarrow NCl_3 + NH_3 \tag{2}$$

$$NHCl_2 + NH_2Cl \rightarrow N_2 + 3H^+ + 3Cl^- \tag{3}$$

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.

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[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 $NH_2Cl$ ($\lambda_{max} = 251$ 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

$$NCT + NH_4^+ \leftrightarrow NH_2Cl + Tau \tag{4}$$

is $K_{NCT/NH_4} = [NH_2Cl][Tau]/[NCT]/[NH_4^+]/f_a^2$, with $f_a$ denoting the activity coefficient.

The equilibrium concentrations $NH_2Cl$, NCT and NDCT were determined photometrically (see above), while $[NH_4^+]$ was found by the equations:

$$c(NH_4Cl) = [NH_4^+] + [NH_3] + [NH_2Cl] \quad \text{and}$$

$$K_{NH_3/NH_4^+} = \frac{[NH_3]aH^+}{f_a[NH_4^+]}$$

giving

$$[NH_4^+] = (c(NH_4Cl) - [NH_2Cl])/(1 + f_aK_{NH_3/NH_4^+}/aH^+)$$

Because the $NH_2Cl$ formation according to Eq. (4) is superimposed by disproportionation of NCT (Gottardi and Nagl, 2002) (see Eq. (7)) the calculation of [Tau] was based on the mass-balances Eqs. (5) and (6) which include also NDCT:

$$c(NCT) = [NCT] + [NDCT] + [Tau] \qquad (5)$$

$$c(NCT) = [NH_2Cl] + [NCT] + 2[NDCT] \qquad (6)$$

There exist two variants for defining [Tau]:

(a) From Eq. (5) follows:

$$[Tau] = c(NCT)P - [NCT] - [NDCT]$$

where $P$ regards the iodometric purity of NCT-Na which in general amounts 97–98%.

(b) Combining Eqs. (5) and (6) and assuming that the difference to 100% NCT-Na concerns taurine yields

$$[Tau] = [NH_2Cl] + [NDCT] + c(NCT)(1 - P)$$

Both variants were used for the calculation of the equilibrium constant, termed as $K(a)$ and $K(b)$.

The activity coefficient at 25 °C was calculated after Hückel (Linnet, 1970):

$$f_a = 10^{-0.509J^{0.5}/(1+0.984J^{0.5})}$$

where the ionic strength comes to

$$J = \frac{c(NCT) + c(NH_4Cl) + [NCT] + [NDCT] + [NH_4^+]}{2}$$

*Procedure.* Weighed samples of NCT-Na (iodometric purity 97.55%) and ammonium chloride, each varying between 0.01 and 0.05 M, were dissolved in 50.0 mL bi-distilled water. After keeping for 15 min the pH was read and one part of the solution transferred to a photometric cell for measuring the UV absorptions at 237.0, 270.5, and 302.6 nm. Both measurements were conducted at 25.0 °C.

Based on nine independent experiments the mass-law constants $K(a) = (5.02 \pm 0.61)E-3$ and $K(b) = (6.57 \pm 0.71)E-3$ were found. Since a decision concerning the accuracy of both variants would be speculative, the mean $\pm$ S.D. of each pair

was used which gave an average value of $K_{NCT/NH_4} = (5.8 \pm 1.2)E-3$ which represents a reasonable estimate.

This value differs significantly ($P > 0.999$) from the already published one of $2.0 \pm 0.04E-2$ (Gottardi and Nagl, 2002) which was assayed in the presence of phosphate buffer (see below) and without respecting disproportionation of NCT. It should be replaced by the new value.

### 2.1.6. Calculational estimation of $[NH_2Cl]$

It is based on the molar concentrations of NCT, ammonium chloride, and the pH of the resulting solution. With $K_1 = K_{NCT/NH_4}$, $K_2 = K_{NH_3/NH_4^+}$, $C = c(NCT-Na)$, $D = c(NH_4Cl)$, $x = [NH_2Cl]$, $[NCT] = c(NCT)-[NH_2Cl]$, $[Tau] = [NCT]$, and the above definitions for $[NH_4^+]$ and $f_a$ follows the polynomial

$$x^2(1 - K_1 + K_2/aH^+) + K_1x(C + D) - K_1CD = 0$$

which allows to calculate $[NH_2Cl]$.

The parameter $aH^+$ was found with

$$pH = A_0 + A_1c(NCT) + A_2c(NH_4Cl)$$

The constants $A_0 = 6.555$, $A_1 = 17.227$, $A_2 = -3.096$ were assessed by linear multiple regression of experimental data. The correlation coefficient $r^2 = 0.93$ revealed an acceptable congruence with the actual values.

*Annotation.* This algorithm does not consider disproportionation why the term [NDCT] was omitted. It concerns therefore the initial equilibrium concentration of $NH_2Cl$.

### 2.1.7. Preparation of antiseptic NCT-Na + ammonium chloride formulations

The 0.1–1.0% NCT-Na ($5.5E-3$ to $5.5E-2$ M) and 0.01–0.1% ammonium chloride ($1.87E-3$ to $1.87E-2$ M) were dissolved in water without a buffer and stored in the refrigerator (1–3 °C). Under these conditions an adequate antiseptic activity for at least 1 month was observed.

### 2.1.8. Preparation of a pure monochloramine solution

The 50 mL of an aqueous solution of 1% NCT-Na (55 mM) and 0.3% ammonium chloride (56 mM) was distilled in a rotary evaporator (water jet vacuum, bath temperature 50 °C) until dryness. The UV-spectrum of the distillate (45 mL, pH 9.5) showed one single peak at $\lambda_{max} = 243.8$ nm typical for monochloramine with $A_{243.8} = 0.3557$. The molar absorption coefficient was assessed iodometrically, giving $\varepsilon_{243.8} = 461.6 \pm 1.9$ L mol$^{-1}$ cm$^{-1}$ ($N = 7$) which corresponded well with published data, e.g., $\lambda_{max} = 244$ nm and $\varepsilon = 458$ L mol$^{-1}$ cm$^{-1}$ (Ferriol et al., 1990). Stored in the refrigerator the distilled solution ($c(NH_2Cl) = 7.71E-3$ M resp. 397 ppm) was fairly stable with a daily decrease of $\approx 0.6\%$.

#### 2.1.8.1. Assessing the $NH_2Cl$ capacity. The distillation procedure turned out appropriate for characterizing NCT-Na/ammonium chloride mixtures. In contrast to the UV-method (see above) which gives the equilibrium concentration it reveals the available $NH_2Cl$ capacity.

A defined volume ($V_1$) of the aqueous solution was distilled until dryness under the quoted conditions. From $A_{243.8}$ and the

volume ($V_2$) of the distilled fraction the evaporated moles of monochloramine were found with

$$\text{mol NH}_2\text{Cl} = V_2 \frac{A_{243.8}/\varepsilon}{1000}$$

while the monochloramine capacity per mL of the tested solution was

$$\mu\text{mol NH}_2\text{Cl} = \frac{V_2}{V_1} \frac{A_{243.8}}{\varepsilon} \times 1000$$

### 2.2. Microbiological experiments

#### 2.2.1. Bacteria

Bacterial strains, *Staphylococcus aureus* ATCC 25923 and 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 11229, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 27853, and a clinical isolate of *Klebsiella marcescens* deep-frozen for storage were grown overnight on tryptic soy agar (Merck, Darmstadt, Germany). Colonies from this agar were grown in tryptic soy broth (Merck, Darmstadt, Germany) at 37 °C overnight and washed twice with saline.

#### 2.2.2. Killing tests

Bacteria were diluted in buffered test solution to concentrations of $1 \times 10^6$ to $5 \times 10^7$ CFU/mL. Immediately and subsequent to different incubation times at room temperature (RT), aliquots were removed, and NCT with or without ammonium chloride or plain monochloramine was inactivated by 10-fold dilution in 0.3% sodium thiosulfate. Aliquots (50 μL) of these solutions as well as of 100-fold further dilutions in saline were spread onto tryptic soy agar plates with an automatic spiral plater (Don Whitley Scientific Limited, West Yorkshire, UK) in duplicates allowing a detection limit of 200 CFU/mL. Plates were grown at 37 °C, and CFU were counted after 24 and 48 h. Controls without NCT were treated the same way. Ammonium chloride as well as NCT or NCT plus ammonium chloride inactivated with sodium thiosulfate *before* the addition of bacteria had no influence on viability as shown in preliminary experiments.

### 2.3. Statistics

Student's *t*-test was used for comparison of paired means of two groups of measurements. One-way analysis of variance and Dunnett's multiple comparison test (Graphpad Software Inc.) were applied for evaluation of more than two groups of measurements. *P* values of <0.05 were considered significant.

## 3. Results

### 3.1. Reaction of active chlorine compounds with $NH_4^+$ at room temperature

If chloramine-T (CAT) was used as chlorinating agent, the reaction did not stop at monochloramine. Even in the presence of excess ammonium chloride the higher chlorinated derivatives di- and trichloramine, $NHCl_2$ and $NCl_3$, were formed, whereby the pH dropped from 6.8 to 2.9 within 8 min. The underlying reactions are shown in Eqs. (1)–(3). If the same experiment was done in the presence of 0.1 M phosphate buffer, the pH dropped from 7.0 only to 6.3 within 2 h. However, decomposition under liberation of nitrogen gas and a 90% loss of oxidation capacity occurred within 1 h. Calcium hypochlorite and sodium dichloro-isocyanurate behaved in the same manner. Contrary to the quoted active chlorine compounds, NCT reacted with ammonium salts only to the stage of monochloramine, which means that the unwanted by-products $NHCl_2$, $NCl_3$, and $N_2$ were not formed and acidification by HCl formation (see Eq. (3)) largely stayed away. In the aqueous solution of NCT and ammonium chloride, therefore, Eq. (4) represents the main reaction whose equilibrium settled within 10–15 min. As a side-reaction disproportionation of NCT has to be considered (see below).
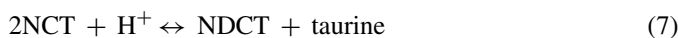
### 3.2. Stability

#### 3.2.1. pH-effects in fresh NCT-Na/ammonium chloride solutions

As a result of lack of buffer the initial pH showed no defined value but ranged within 6.0–8.0 if the concentrations of each NCT and ammonium chloride varied between 0.01% and 1.0%. This pH range, by all means, is compatible with the use in medical practice, all the more the mixture has no buffering potency and therefore adapts to the prevailing buffered system.

The initial pH changed by the time, whereby the starting value was predetermined by the concentrations of both components. 1% NCT (0.055 M) in the presence of 0.01, 0.1 and 1.0 M ammonium chloride showed an initial pH of 7.92, 7.03, and 6.19, respectively. In case of 0.01 M ammonium chloride it dropped within 0.5 h to 7.81, while it increased in 0.1 and 1.0 M ammonium chloride to 7.20 and 6.58, respectively. Long-term (more than 2 months) equilibrium pH values at 1–3 °C are shown in Table 1. They disclose a change of only +0.2, +0.3, and −0.3 pH units for 1% NCT in the presence of 0.02%, 0.1%, and 0.5% ammonium chloride, respectively.

#### 3.2.2. Development of NDCT

UV measurements uncovered that besides the formation of monochloramine (Eq. (4)) disproportionation to NDCT (Eq. (7)) as a side-reaction becomes important (Gottardi et al., 2005) whose rate increases with the concentration of ammonium chloride, an effect which can be attributed to its acidifying property ($NH_4^+$: p$K$a = 9.246):

$$2\text{NCT} + \text{H}^+ \leftrightarrow \text{NDCT} + \text{taurine} \tag{7}$$

In the absence of a buffer (see below) this reaction runs much slower than Eq. (4). In mixtures of 1% NCT (0.055 M) with 0.01, 0.1 and 1.0 M ammonium chloride after 1 h levels of <1E−5, 1.7E−4, and 9.2E−4 M NDCT were found, respectively. Twice these values equal the connected decrease of NCT of <0.04%,

Table 1
Change of pH, $c$(Ox), and NH$_2$Cl capacity[a] of 1% NCT solutions spiked with 0.02%, 0.10% and 0.5% ammonium chloride after a period of 2 months in the refrigerator (1–3 °C)

| $c$(NH$_4$Cl) | 0.02% | | 0.1% | | 0.5% | |
|---|---|---|---|---|---|---|
| Age of solution (days) | 0 | 67 | 0 | 67 | 0 | 67 |
| pH | 8.10 | 8.30 | 7.70 | 8.05 | 7.30 | 7.61 |
| $c$(Ox) (mol/L) | 5.50E−2 | 5.36E−2 | 5.50E−2 | 4.93E−2 | 5.50E−2 | 4.30E−2 |
| $\Delta$ | | −2.5% | | −10.3% | | −21.8% |
| NH$_2$Cl-capacity (μmol/mL) | 0.70 | 0.25 | 2.24 | 0.98 | 8.08 | 2.01 |
| $\Delta$ | | −64.3% | | −56.3% | | −75.1% |

[a] Assessed by vacuum distillation.

0.6%, and 3.3%. Via this decrease of NCT disproportionation is also responsible for the initial decrease of monochloramine (see Fig. 1). On the whole, however, disproportionation is no serious flaw, because it regulates the pH by absorbing protons (see Eq. (7) and Section 4).

Long-term monitoring of a solution containing 0.5% NCT and 0.25% ammonium chloride revealed that the equilibrium of disproportionation (Eq. (7)) was not completely attained even after 50 days (Fig. 1). In the presence of phosphate buffer, however, this equilibrium settled much faster and was reached after ≈1 day with 0.05 M and after ≈1 h with 0.5 M phosphate buffer, respectively. These findings complied with published data concerning the disproportionation of NCT which is promoted not only by a more acidic environment (Gottardi and Nagl, 2002), but also by the buffer concentration (Gottardi et al., 2005).

Additionally, phosphate buffer provoked also a significant boost of loss of $c$(Ox) which correlated with buffer concentration as well (Fig. 2). The origin of this effect is the redox-reaction Eq. (3) whose rate is connected with the buffer-sensitive disproportionation Eq. (1) (Valentine et al., 1988) which enables the $c$(Ox) consuming redox-reaction Eq. (3).

### 3.2.3. Consequences for formulating use solutions

Based on these results the NCT-Na/ammonium chloride formulation must not contain a buffer, all the more the pH changes only in a low extent at cooled storage. Notwithstanding general practice in formulating antiseptic preparations the use of a buffer (and any other additive) was waived, therefore.
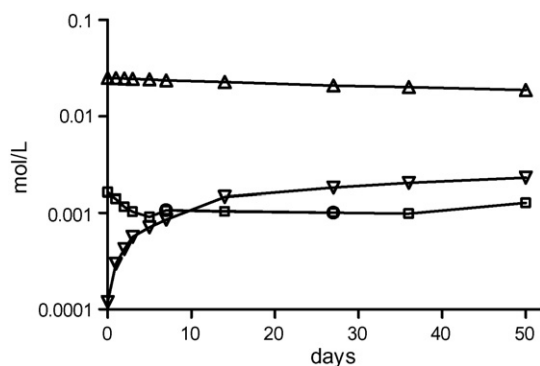
### 3.3. Calculated monochloramine concentration in NCT/ammonium chloride mixtures

The presented algorithm enables us to tailor the disinfecting power of the preparation to the particular site of application and/or kind of microorganism. From Fig. 3 can be derived that with combinations of NCT and ammonium chloride, each in the range of 0.01–1.0%, an equilibrium concentration of 5–254 ppm can be attained, the upper limit representing a very powerful formulation (see also Table 2).
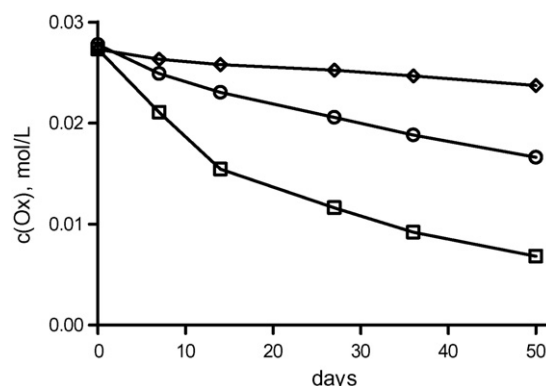
Fig. 2. Influence of buffer concentration on the stability of 0.5% NCT (0.0275 M) and 0.25% NH$_4$Cl (0.0467 M) upon storage at 2–4 °C, $c$(Ox) measured by iodometric titration. (◇) Without buffer; (○) 0.05 M phosphate; (□) 0.5 M phosphate.

Fig. 1. Course of [NH$_2$Cl], [NCT], and [NDCT] in 0.5% NCT (0.0275 M) and 0.25% NH$_4$Cl (0.0467 M) upon storage at 2–4 °C without buffer, equilibrium concentrations measured by UV-photometry. (△) [NCT]; (□) [NH$_2$Cl]; (▽) [NDCT].
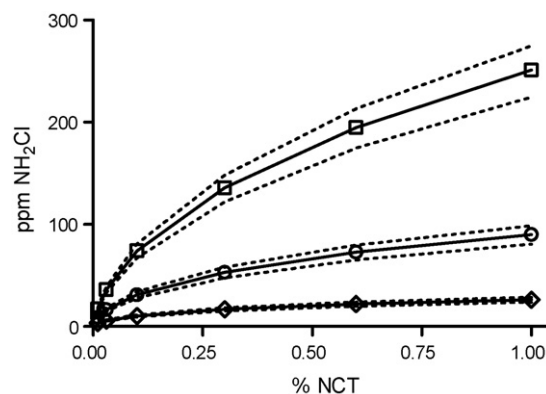
Fig. 3. Equilibrium concentrations of NH$_2$Cl in aqueous solutions of NCT in the presence of ammonium chloride, calculated with $K_{NCT/NH_4} = (5.8 \pm 1.2)E{-}3$. Concentration of ammonium chloride: (◇) 0.01%; (○) 0.1%; (□) 1.0%. Dotted lines: mean values ± S.D.

Table 2
Initial equilibrium concentrations of NCT and $NH_2Cl$ in formulations suggested for practice[a]

| $c(NCT)$ (%) | $c(NH_4Cl)$ (%) | $[NH_2Cl]$ (ppm) | $[NCT]$ (mol/L) | $[NH_2Cl]$ (mol/L) | $[NCT]/[NH_2Cl]$ |
|---|---|---|---|---|---|
| 1.0 | 1.0 | 254 | 5.0E−2 | 4.9E−3 | 10 |
| 1.0 | 0.5 | 192 | 5.1E−2 | 3.7E−3 | 14 |
| 1.0 | 0.1 | 91 | 5.3E−2 | 1.8E−3 | 30 |
| 1.0 | 0.01 | 27 | 5.5E−2 | 5.2E−4 | 105 |
| 0.1 | 0.01 | 11 | 5.3E−3 | 2.1E−4 | 26 |
| 0.01 | 0.01 | 4.8 | 4.8E−4 | 6.7E−5 | 7 |

[a] Calculated with $K_{NCT/NH_4} = 5.8E − 3$.

### 3.4. Microbicidal activity

Compared to NCT without additives, NCT plus ammonium chloride demonstrated a distinguished enhancement of bactericidal activity about 200–300-fold. The 0.1% NCT plus 0.1% ammonium chloride reduced the viability of *S. aureus* ATCC 25923 significantly within 1 min, and no more viable bacteria could be detected after 5 min (Fig. 4; detection limit 200 CFU/mL which equals $2.30 \log_{10}$, see Section 2.2.2). Further elevation of the ammonium chloride concentrations (Fig. 4) and the NCT concentration (data not shown) did not lead to significantly more rapid killing. The impact of ammonium chloride on the microbicidal activity of NCT could be confirmed in several bacterial strains (Fig. 5).

### 4. Discussion

#### 4.1. NCT as an ideal chlorinating agent for the system $NH_3/NH_4^+$

In terms of chlorinating and accordingly oxidizing activity, NCT was judged as the least one amongst accessible active chlorine compounds (Gottardi and Nagl, 2002).
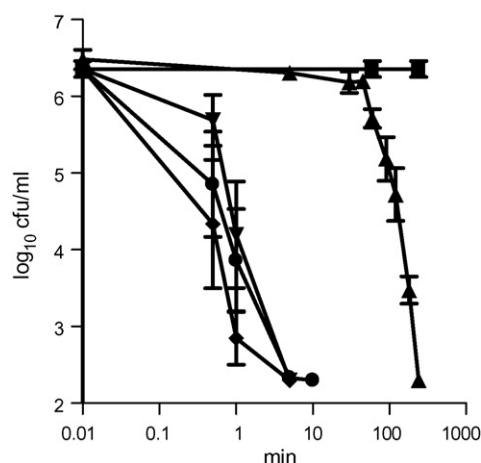


Fig. 4. Killing of *S. aureus* ATCC 25923 by 0.1% NCT alone (▲), 0.1% NCT + 0.1% $NH_4Cl$ (▼), 0.1% NCT + 1% $NH_4Cl$ (♦), 0.1% NCT + 10% $NH_4Cl$ (●) at 20 °C and pH 7.1. Controls without NCT (■). Mean values ± S.E.M. of three to five independent experiments. $P < 0.01$ between NCT + 0.1–10% $NH_4Cl$ and NCT alone; $P > 0.05$ between 0.1% and 10% $NH_4Cl$.

This property is responsible for the absence of dichlor- and trichloramine formation in equilibration with ammonium at room temperature, while stronger oxidants need low temperatures ($-15$ °C (Delalu et al., 2004)) to accomplish the same.

#### 4.2. pH-effects in fresh $NCT/NH_4^+$ mixtures

There can be specified four reactions which chiefly influence the pH.

(1) Hydrolysis of *N*-chlorotaurine: $NCT + H_2O \leftrightarrow NCTH^+ + OH^-$.

As a consequence, plain aqueous NCT exhibits an alkaline reaction which manifests itself initially by a $pH \approx 9.2$ for 1% NCT. Mainly by the action of airborne $CO_2$ it decreases and settles at $\approx 8.1$ after 5 h (Gottardi and Nagl, 2002).

(2) Hydrolysis of ammonium: $NH_4^+ + H_2O \leftrightarrow NH_3 + H_3O^+$.

Because of this acidifying reaction (1.0, 0.1 and 0.01 M ammonium chloride produces a pH of 4.6, 5.1, and 5.6) the presence of ammonium chloride shifts the pH to lower values. Thus, the equilibrium pH of 1% NCT is shifted to 7.5–6.9 in the presence of 0.01–1.0% ammonium chloride.

(3) Disproportionation of NCT (see Eq. (7)).

This reaction has two effects. (i) NCT is transformed to NDCT whose less readiness to react with $NH_4^+$ is known (Gottardi et al., 2005), and (ii) protons are absorbed, why the pH is shifted to higher values.

(4) Decomposition of $NH_2Cl$ (see Eqs. (1)–(3)).

Because the oxidation capacity of $NCT/NH_4^+$ solutions decreases faster than that of plain NCT solutions (0.5% NCT + 0.25% ammonium chloride: 0.32 %/day (see also Fig. 2), 1% NCT: 0.03%/day (Gottardi and Nagl, 2002) both at 0–3 °C), a slight decomposition of $NH_2Cl$ still has to be considered which is associated with acidification (see Eqs. (1)–(3)).

Reactions 4.2 (1)–(4) sufficiently explain the quoted pH-effects: while (1) and (2) are responsible for the initial pH, its time course is regulated by (3) and (4). Since the pH remains rather stable, acidification because of (4) is compensated for by the proton absorbing effect of (3).
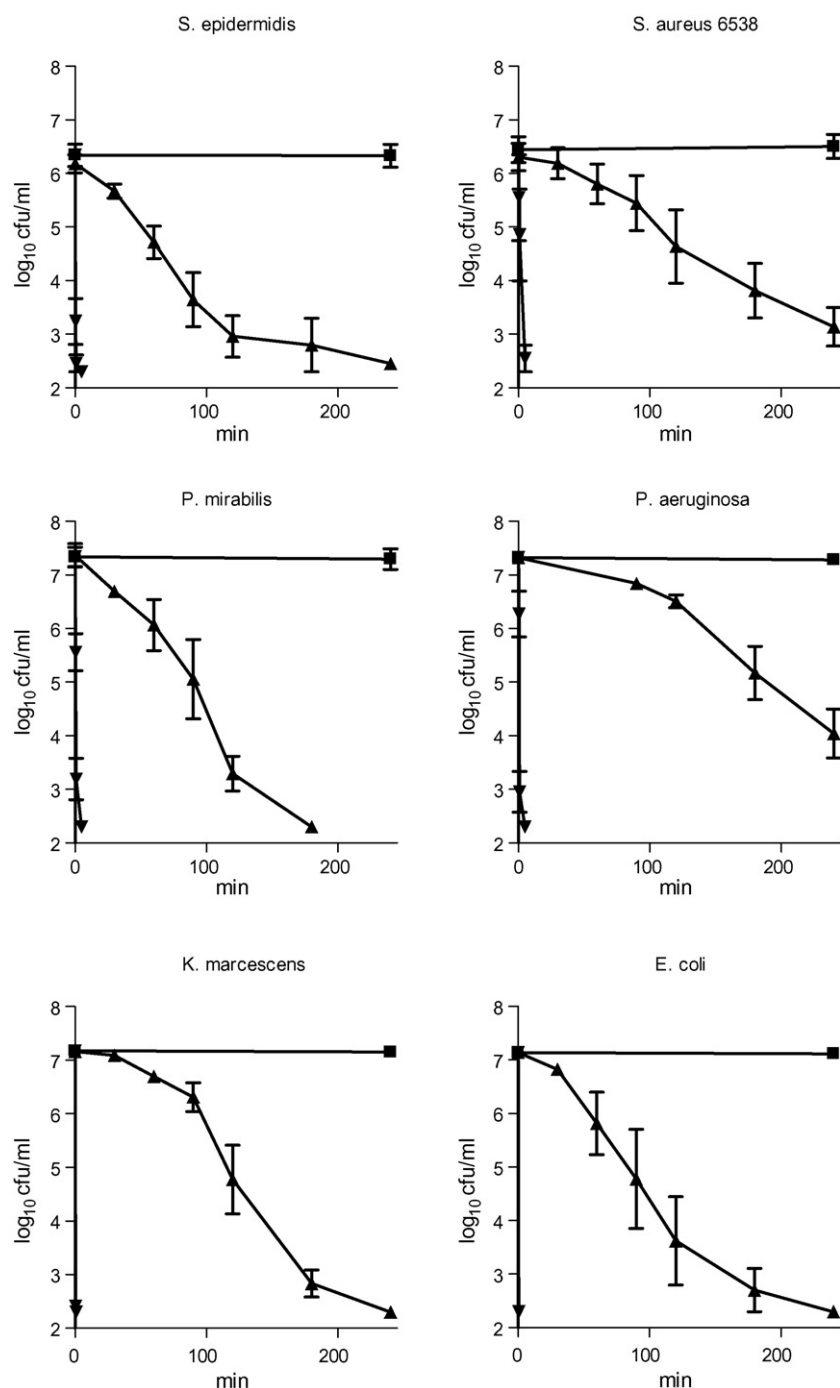
Fig. 5. Killing of six bacterial strains by 0.1% NCT + 0% NH$_4$Cl (▲), and 0.1% NCT + 0.1% NH$_4$Cl (▼) at 20 °C and pH 7.1. Controls without NCT (■). Mean values ± S.E.M. of four to five independent experiments. $P < 0.01$ between all curves in all figures.

### 4.3. Stability

The above exemplified reactions help to understand the two features of stability. One concerns the loss of oxidation capacity as discussed in (4.2.4), while the other concerns the conversion of NCT into NDCT, according to (4.2.3i), which is not connected with a loss of $c(Ox)$ but effects a decrease of monochloramine, too (Fig. 1). Though (4.2.3i) might be a slight flaw, (4.2.3ii) turns out as a beneficial feature because it brings about a sort of dynamic pH regulation responsible for the unexpected stability

of NCT/NH$_4^+$ mixtures. A special role plays phosphate buffer which accelerates the disproportionation of both NCT (Eq. (7)) and monochloramine (Eq. (1)) and, therefore, impairs stability.

### 4.4. The highly bactericidal potential of NH$_2$Cl

The equilibrium constant ($K_{NCT/NH_4} = 0.0058$) suggests that the reaction between NCT and NH$_4^+$ (Eq. (4)) is far away from being quantitative, which as a first approximation points to a higher oxidative power of NH$_2$Cl. However, this fact is

Table 3
$NH_4^+$ concentration in body fluids (Ciba-Geigy, 1983) and equilibrium concentrations of $NH_2Cl$[a] after equilibration with 1%, 0.1% and 1E−5 M NCT

| Body fluid | $c(NH_4^+)$ (mmol/L) | $NH_2Cl$ (ppm) | | |
|---|---|---|---|---|
| | | 1% NCT | 0.1% NCT | 1E−5 M NCT |
| Tears | 3 | 35 | 14 | 0.4 |
| Liquor cerebrosp. | 0.155 | 5 | 3 | 0.1 |
| Gastric juice | 1.02 | 19 | 8 | 0.3 |
| Saliva | 0.12–0.4 | 4–10 | 2–5 | 0.1–0.2 |
| Sudor | 3 | 35 | 14 | 0.4 |
| Urine | 21[b] | 97 | 33 | 0.5 |
| Semen | 1.2 | 21 | 9 | 0.3 |
| Nasal mucus (Nagl et al., 2001) | 1.1 | 20 | 8 | 0.3 |

[a] Calculated with $K_{NCT/NH_4} = 5.8E − 3$.
[b] Calculated from 42.7 mmol/day for a daily volume of 2 L of urine.

not the only reason for its superiority in killing microorganisms. Chloramine-T (CAT), though being a stronger oxidant than $NH_2Cl$, is less bactericidal. Killing of *E. coli* with 3.55E−4 M CAT (0.01%) needed 5 min, while with the same concentration of $NH_2Cl$ it was only 1 min (M. Nagl, unpublished). Regarding oxidizing potency the sequence CAT > $NH_2Cl$ > NCT applies, while it is $NH_2Cl$ > CAT > NCT concerning bactericidal activity.

The superiority of $NH_2Cl$ roots in the small molecular bulk (the molar weights for $NH_2Cl$, NCT, and CAT come to 51.5, 181.6, and 281.7) and the absence of a charge. NCT as well as CAT are much bigger, and both are sodium salts and therefore present as anions in an aqueous system. The difference between CAT and NCT, on the other hand, is grounded on the higher oxidizing potency of CAT. From these results can be concluded that small molecular bulk and absence of charge are outweighing oxidizing potency.

These considerations point out that the active agents NCT and $NH_2Cl$ occurring in NCT-Na/ammonium chloride mixtures possess varying bactericidal reactivity. The results of relevant calculations in Table 2 disclose that in formulations suggested for practice, [NCT] outbalances [$NH_2Cl$] by a factor of 10, at least. Notwithstanding its low equilibrium concentration $NH_2Cl$ induces a tremendous increase of microbicidal activity.

## 4.5. NCT/ammonium chloride formulations in practice

### 4.5.1. Appropriate mix ratios

Hitherto experiences with plain NCT solutions proved 1% and 0.1% as suitable concentrations in several clinical studies (Nagl et al., 2000, 2003; Neher et al., 2004, 2005; Romanowski et al., 2006; Teuchner et al., 2005). They should be the starting concentrations for NCT/ammonium chloride formulations which are completed with an appropriate concentration of ammonium chloride (Table 1). NCT concentrations lower than 0.1% are not recommended because of a too low oxidation capacity. Since disinfection processes with active halogen agents are always associated with consumption (i.e. reduction) of $c(Ox)$, an adequate reservoir of NCT enables the re-formation of $NH_2Cl$ (Eq. (4)), which, because of its higher reactivity, will be used up first of all.

### 4.5.2. Tolerance

In vivo studies with plain NCT showed similarly good tolerability in the rabbit and human eye (Nagl et al., 1998a, 2000). In vivo tests with mixtures of 1% NCT and 0.1% ammonium chloride, yielding [$NH_2Cl$] ≈ 91 ppm revealed a good tolerance and efficacy in adenoviral conjunctivitis in the rabbit eye [(Romanowski et al., 2006) and M. Nagl, unpublished]. At the normal skin higher concentrations are possible: a patient suffering from a fungal infection was treated with a 1%/1% mixture (254 ppm $NH_2Cl$) which caused a marked burning. However, healing was achieved within only 2 days (M. Nagl, R. Arnitz, unpublished).

## 4.6. Increase of bactericidal activity of NCT caused by body-own $NH_4^+$

In Table 3 are listed the $NH_4^+$ concentrations of several body fluids and calculated monochloramine concentrations after equilibration with 1%, 0.1%, and 1E−5 M NCT, the latter being an estimate of body-own NCT emerging at sites where phagocytosis occurs (Weiss et al., 1982; Grisham et al., 1984). The calculated values help to understand the increasing bactericidal activity of NCT in the presence of body fluids (Nagl and Gottardi, 1996; Nagl et al., 2001). Because of the high $NH_4^+$ concentration in urine a significant increase of bactericidal activity of NCT can be expected (calculated: 97 ppm $NH_2Cl$). This was actually observed in treatment of urinary infections caused by omniresistant *P. aeruginosa* (Nagl et al., 1998b).

## 4.7. Monochloramine as a natural agent

The state of NCT as an endogenous compound is undisputed since it is produced during the innate defense of infections in human granulocytes and monocytes. Ammonium, on the other hand, as Table 3 shows, is an ubiquitous component of body fluids. This allows to consider the reaction product $NH_2Cl$ as an endogenous compound (Thomas, 1979; Grisham et al., 1984), too, and supports in recommending the *N*-chlorotaurine/ammonium chloride formulation for application in man.

## 4.8. Conclusion

The combination of *N*-chlorotaurine sodium and ammonium chloride in plain aqueous solution represents a powerful antiseptic preparation sufficiently stable for a topical therapy of infections. Its outstanding microbicidal properties are founded in the formation of monochloramine which is distinguished by the absence of dichloramine and trichloramine.

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