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Chemical Properties of *N*-Chlorotaurine Sodium, a Key Compound in the Human Defence System

N-Chlorotaurine (NCT) is known to play an important role in the human defence system. The already proved utility of the sodium salt as a disinfectant in human medicine suggested a thorough investigation of its chemical properties. Chlorine transfer to N-H groups (transhalogenation) and oxidation of thio and aromatic compounds represent its main reactions. Auto-chlorination causes disproportionation forming *N,N*-dichlorotaurine (NDCT) with K_d = [NDCT][taurine]/f_a[NCT]^2 aH^+ = (4.5 ± 0.8) × 10^6, while the reaction with ammonium releasing NH_2Cl is characterised by K_{NHCl2} = [NH_2Cl][taurine]/[NCT][NH_4^+] f_a^2 = 0.02 ± 0.004. The verified unique stability and low-level reactivity of NCT are considered essential for its function in the mammalian defence system and its practical applicability, which manifests itself in an optimal compromise between microbicidal activity and toxicity.

Keywords: *N*-Chloroamino acids; Transhalogenation; Antimicrobial agent; Endogenous long-lived oxidant; Non-toxic disinfectant

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Introduction

Active chlorine compounds (containing –O–Cl or >N–Cl functions) are known to act as oxidants with bactericidal properties. While O-Cl compounds used in practice are limited to salts of HOCl (hypochlorites), a variety of N-Cl compounds are in use, chiefly *N*-chloroamides and -imides, for instance chloroisocyanuric acids and their salts, chloramine T, 1,3-dichloro-5,5-dimethylhydantoin. *N*-Chloroalkylamines (e. g. CH_3NHCl), on the other hand, are of no importance in practice because of their unpleasant smell and low stability. Even less stable are the *N*-chloroamino acids which, except *N*-chlorotaurine (NCT), are available only in solution.

However, the free *N*-chloroamino acids attracted increasing interest when their role in the human defence system was detected, where they act as vehicles for the oxidation capacity of the highly cytotoxic hypochlorous acid formed during the "oxidative burst" in granulocytes and monocytes [1, 2]. NCT plays a major role in this regard, on the one hand because taurine is present in relatively high concentrations in these cells and, on the other hand, because of its comparatively high stability [3, 4]. The assumed biological function of NCT is first the termination of the inflammatory process, since it decreases the production of pro-inflammatory cytokines, and secondly a contribution to the inactivation of pathogens [2, 5, 6]. The first reference to NCT[a] was given by Stelmaszynska and Zgliczynski [7] who produced the compound from a mixture of taurine, hydrogen peroxide, sodium chloride, and myeloperoxidase and proved its identity by spectrophotometry, chromatography, and the $Na^{36}Cl$ isotope technique. Further evidence has been gained by ^{13}C-NMR spectroscopy, showing that taurine is transformed in aqueous solution to NCT by hypochlorous acid [8]. However, this synthetic route failed because it was not possible to isolate the compound from the aqueous solution. It finally succeeded in an alcoholic system where the pure sodium salt could be prepared [9].

The aim of this paper is to present the results of investigations on the pure compound NCT-Na which includes also reviewing its properties and comparing them with some features observed for hypochlorite in the presence of surplus taurine. Considering its function in innate immunity and its proposed use as a disinfectant in human medicine, a thorough understanding of the pure compound seems to be mandatory.

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[a] 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 sodium salt is specified by NCT-Na.

Full Paper

Results

Properties of the sodium salt

NCT-Na forms column like spears (re-crystallised from methanol/ethanol) which decompose at 130–135 °C. Long-term tests reveal an outstanding stability compared to other *N*-chloroamino acids which exist only in solution and for a short time [9]. Upon storage at 2–4 °C the salt loses only 10 % of its oxidation capacity during one year, while the same decrease needs approximately two years if it is kept at –20 °C (Figure 1a).

The loss of oxidation capacity is connected with the appearance of a nitrile band at 2265 cm^{-1}. The reaction is not catalysed by moisture as could be observed with a specimen thoroughly purified by re-crystallisation and kept in an evacuated and sealed glass-bulb. After 3.5 years at room temperature without light protection the oxidation capacity had completely vanished though the crystals exhibited no macroscopic alteration. Since taurine (by re-crystallisation from water) and cyanomethane-sulfonic acid[b] (by extraction with hot methanol) could be isolated from the strongly acidic product, a decomposition according Eq. (1) seems plausible (see also "Discussion").

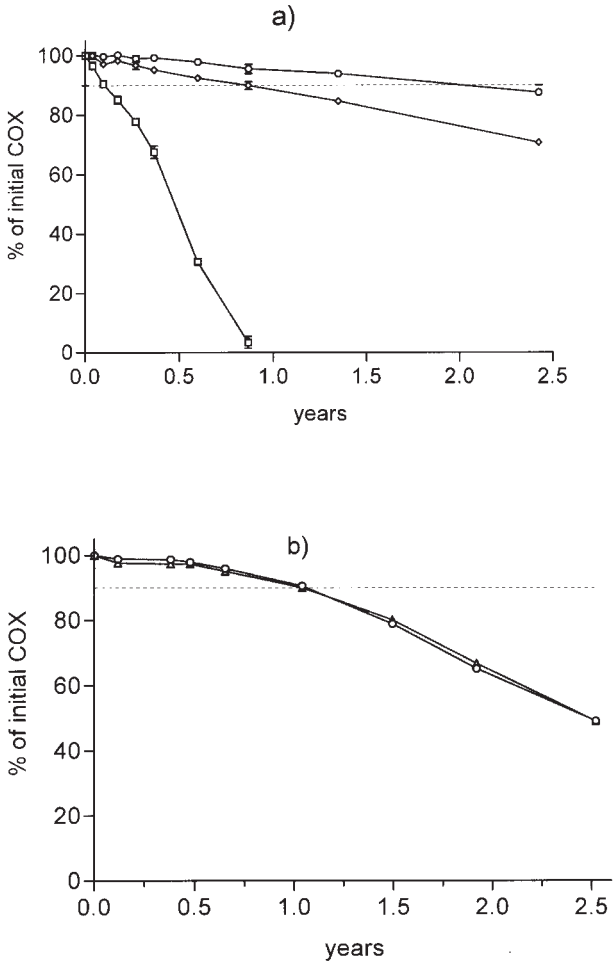


Figure 1. Stability of NCT (mean values of three measurements). (a) crystalline NCT-Na at room temperature (-□-), 2–5 °C (-◇-) and -20 °C (-○-); the difference between all curves was significant (mean values ± S.D., $P < 0.01$, *t* test). (b) 0.75 % (*w/v*) aqueous solution of crude (-○-) and recrystallised NCT-Na (-△-) without buffer at 2–5 °C; $P > 0.05$ (*t* test). Variation coefficients ≤ 1.5 % (not shown).

$$2\ ClHNCH_2CH_2SO_3Na \rightarrow H_3N^+CH_2CH_2SO_3^- + N{\equiv}C\text{-}CH_2\text{-}SO_3H + 2\ NaCl \quad (1)$$

NCT-Na is readily soluble in water (1.4 g/mL) and dimethyl sulfoxide (0.43 g/mL), poorly soluble in alcohols (methanol: 0.04 g/mL, ethanol: 0.0076 g/mL), and insoluble in apolar solvents. The aqueous solution of NCT, too, is distinguished by an outstanding stability showing a decomposition rate of only 0.43 %/day at room temperature which declines to ≈0.03 %/day and 10 %/year, respectively, if kept at 2–4 °C (Figure 1 b). These results are derived from iodometrically assayed loss of oxidation capacity at the equilibrium pH 8.2 (see below). Based on the absorbance at 250 nm, Antelo et al. [11] reported a decrease of less than 1 % after 100 hours (< 0.24 %/day) at pH 9.31, however, without indicating the temperature of storage.

The dimethyl sulfoxide solution, on the other hand, turned out to be less stable with a decrease of oxidation capacity amounting to ≈11 % per day at room temperature, which is in contrast to previous statements that chloramines in aqueous solution would not react with dimethyl sulfoxide [12, 13].

Spectra

IR spectrum: The introduction of one chlorine atom gives rise to a considerable simplification compared to the spectrum of taurine, mainly in the N-H region where NCT-Na shows only one sharp band at 3260 cm^{-1} typical for secondary amines (Figure 2). Both spectra were recorded with KBr pellets.

UV Spectrum[c]: NCT-Na displays one peak at 251 nm which is characteristic for the chromophore NHCl (Fig-

[b] A reference sample of cyanomethane-sulfonic acid (ν_{CN} = 2266 cm^{-1}) was prepared from chloroacetonitrile and sodium sulfite [10].

[c] The UV spectrum of NCT in neutral and acidic solution was first published by Zgliczynski and Stelmaszynska [14] who found peaks at 250 and 300 nm which they assigned to NCT and NDCT, respectively. The peak of NDCT at 206.6 nm, however, was not detected.

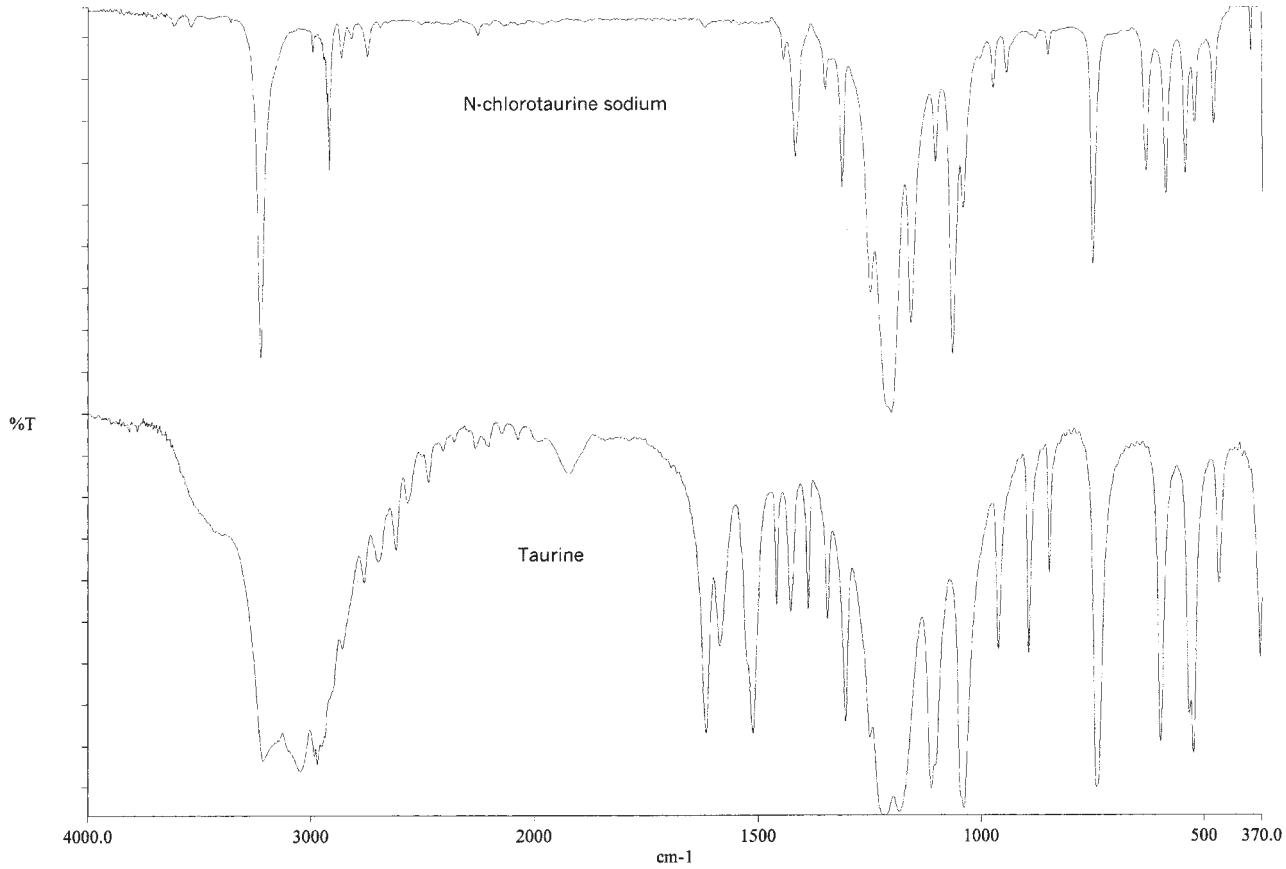


Figure 2. IR spectra of taurine and N-chlorotaurine sodium, 4000–370 cm^{-1} (KBr pellets).

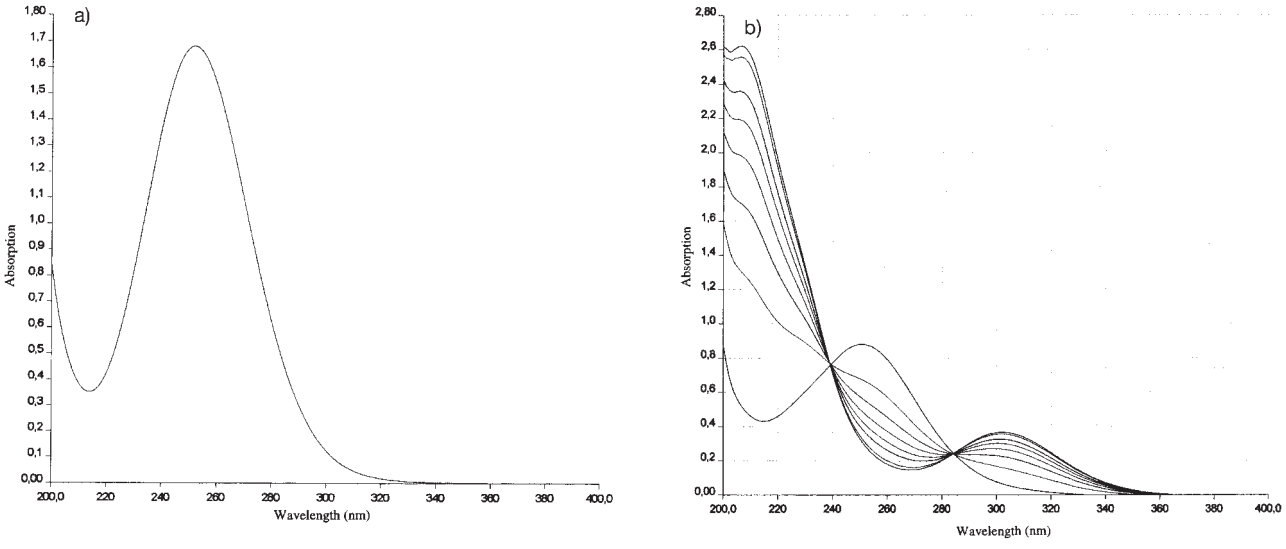


Figure 3. UV spectra of NCT (cell length 0.10 cm). (a) NCT-Na in water (0.04438 M). (b) NCT-Na in 0.5 N H_2SO_4 (0.0219 M); overlay-spectra, scanning time 5 min.

ure 3 a). The exact parameters are λ_{max} = 250.9 nm and ε = 397.4 L mol^{-1} cm^{-1}, while Carr et al. [15] found ε_{252} = 429 L mol^{-1} cm^{-1}. The valley at 215 nm becomes important for verifying the absence of NDCT or other impurities which manifests itself in a ratio of $A_{250.9} : A_{215} > 4$ which is the value for pure NCT. If disproportionation is provoked by addition of acid (see below), overlay-spectra with two isosbestic points at 240.0 and 285.5 nm can be obtained (Figure 3 b). The two peaks at 206.6 and 302.1 nm are characteristic for NDCT. Because of these specific differences, the actual concentrations of NCT and NDCT during disproportionation can be assayed by means of UV spectroscopy (see "Materials and Methods").

Mass spectrum: Using chemical ionisation the most important fragments were HCl^+, $ClH{=}CH_2^+$, SO_2^+, and $NHCH_2CH_2^+$, while the molecular peak was not found. This was possible using electrospray ionisation MS which showed clearly the mass peaks of M^+ and $M^+ - H$ at 181.1 and 180.1 dalton.

^{13}C-NMR spectrum: NCT-Na dissolved in D_2O shows two methylene singlets at 51.8 and 50.0 ppm (Bruker AM 300, operating at 75.47 MHz with dioxane as internal standard), whereas Lin et al. [8] reported a pair of singlets at 53.1 and 51.3 ppm, respectively, showing the same difference of 1.8 ppm. Since the differences of the absolute values can be attributed to the different operating parameters – Lin et al. [8] used an external standard – there is no doubt that we are dealing with the same compound in both cases.

HPLC: In contrast to aqueous solution, a chromatogram with only one peak, indicating absence of the reaction products of disproportionation, was obtained in methanolic solution.

Hydrolysis

Upon dissolution in water an alkaline reaction arises immediately which can be explained by hydrolysis (Eq. (2)).

$$ClHN{-}CH_2{-}CH_2{-}SO_3^- + H_2O \leftrightarrow ClH_2N^+{-}CH_2{-}CH_2{-}SO_3^- + OH^- \quad (2)$$

The initial pH depends on the concentration and ranges between approx. 8.8 and 9.3 in 0.1 % and 1.0 % (*w/v*) solution, respectively. However, the pH immediately begins to decrease and settles at pH 7.1 and 8.2 within 0.2 and 5 h and remains constant for extended periods (Figure 4 a). This equilibration is a result of the acidifying action of airborne CO_2 and the proton consuming disproportionation (see below). The main contribution to acidification comes from CO_2 as the course of pH under nitrogen flushing shows (Figure 4 b). However, there must

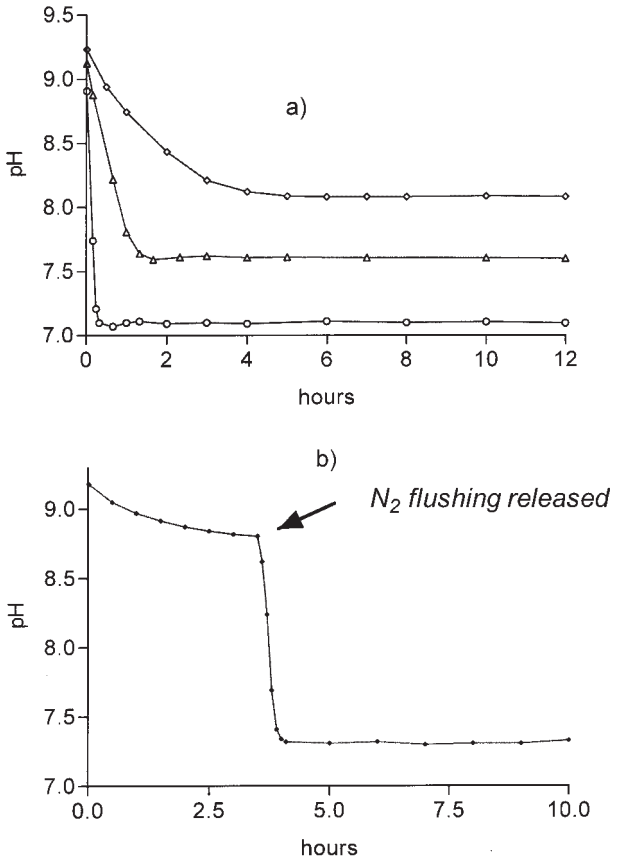


Figure 4. Change of pH in fresh aqueous solutions of NCT-Na. (a) equilibration of 5.85 (-○-), 16.7 (-△-), and 54.6 (-◇-) mM in normal atmosphere. Each point represents a single measurement. $P < 0.01$ (ANOVA). (b) equilibration of 8.6 mM under nitrogen.

also be another origin of pH decrease, probably the following elimination reaction leading to an imine (See also "Discussion").

$$ClHN{-}CH_2{-}CH_2{-}SO_3^- \rightarrow HN{=}CH{-}CH_2{-}SO_3^- + H^+ + Cl^- \quad (3)$$

The extrapolated initial pH of weighed samples of NCT-Na dissolved in CO_2-free H_2O allowed an estimation of the equilibrium constant of Eq. (2) which comes to pK_b = [NCTH$^+$] aOH$^-$/[NCT] = 7.9 ± 0.2 (25 °C).

Disproportionation

NCT equilibrates in aqueous solution by an auto-chlorination forming NDCT and taurine, a reaction which consumes protons (Eq. (4))

$$2\ ClHNCH_2CH_2SO_3^- + H^+ \leftrightarrow Cl_2NCH_2CH_2SO_3^- + H_3N^+CH_2CH_2SO_3^- \quad (4)$$

$$2\ NCT + H^+ \leftrightarrow NDCT + taurine$$

The disproportionation constant was found to be

$$K_d = [NDCT][taurine]/f_a[NCT]^2\ aH^+$$
$$= (4.5 \pm 0.8) \times 10^6\ M^{-1} \quad (5)$$

which was derived from measurements in the pH range of 4.0–7.4. The equilibrium concentration of NCT, calculated with K_d over the whole pH range is shown in Figure 5. Based on NCT solutions made from 0.14 M NaOCl and 0.1 M taurine in the presence of 0.5 M $NaClO_4$, Antelo et al. [11] found a somewhat lower value of $K_d = (1.07 \pm 0.15) \times 10^6\ M^{-1}$.

The position of the equilibrium is influenced by the degree of acidity. The results of kinetic measurements are shown in Table 1, which reveals a second order reaction:

$$\text{rate} = -d[NCT]/dt = k_r\,[NCT]^2\ dm^3\ mol^{-1}\ min^{-1}$$

The observed rate constants are pH dependent and follow the regression line of Eq. (6).

$$\log k_r = (3.83 \pm 0.14) - (0.52 \pm 0.03) \times pH \quad (6)$$

Since they increase substantially with acidity, the participation of the protonated form $NCTH^+$ as the real chlorinating agent (Eq. (7)) seems plausible.

$$NCT + NCTH^+ \leftrightarrow NDCT + taurine \quad (7)$$

The same mechanism was also presumed for the chlorine transfer from inorganic chloramine to amino acids where the active form is NH_3Cl^+ [16].

From the observed second order rate constants the half-reaction times for the disproportionation of NCT solutions can be estimated with $t_{0.5} = k_r^{-1} a^{-1}$ [17] where a denotes the initial concentration. Table 1 shows impressively that the $t_{0.5}$ values decrease with acidity and increase with dilution, ranging from 0.3 s (1 % [*w/v*] NCT, pH 0.3) to 8.0 h (0.1 % [*w/v*] NCT, pH 7.8).

However, it takes much longer to completely establish the disproportionation equilibrium, mainly in a neutral or weakly alkaline medium. Thus, at an initial pH of 7.00 (0.1 M phosphate, final pH 7.35) the equilibrium was attained after approx. 2 h with 2 % NCT (Figure 5b) while $t_{0.5}$ comes to 8 min.

As a result of disproportionation, aqueous solutions of NCT at pH < 8 are always mixtures of the three compounds NCT, NDCT, and taurine. However, at pH ≈ 8.2, which is the equilibrium pH of pure and not buffered 1 % (*w/v*) NCT solution equilibration proceeds very slowly and even after one day at room temperature virtually no NDCT could be detected. In freshly prepared weakly alkaline solutions, therefore, the portion of the reaction products NDCT and taurine can be neglected.

Reaction Eq. (4) is reversible as the disproportionated mixture comproportionates if the pH is raised to neutral

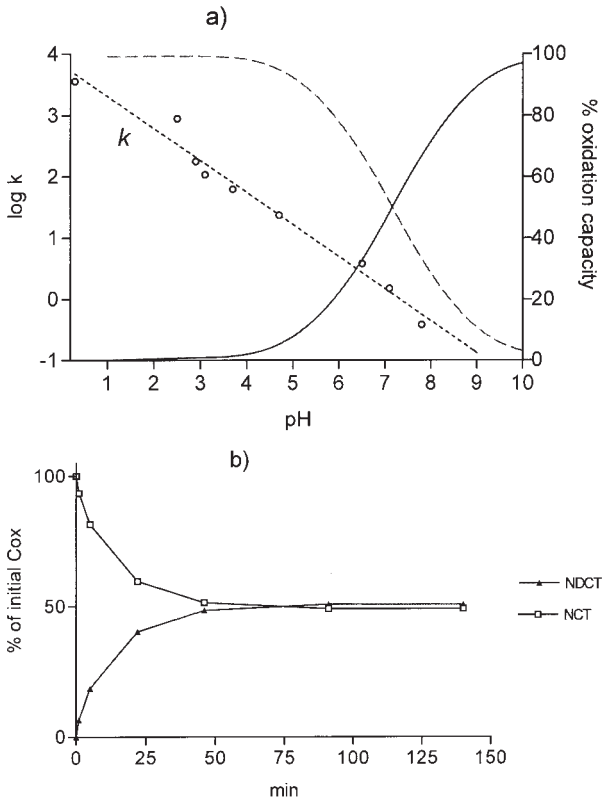


Figure 5. Disproportionation of NCT. (a) Calculated equilibrium concentrations of NCT (—) and NDCT (- - -) as well as reaction rate (○) in dependence on pH. (b) Measured disproportionation of 2 % (*w/v*) NCT in 0.1 M phosphate at room temperature (actual pH: 7.00 → 7.35).

Table 1. Kinetic data of disproportionation at 25.0 °C.

pH	Rate constant k_r $dm^3\ mol^{-1}\ min^{-1}$	Half-reaction time[a] 1.0% NCT	0.1 % NCT	r[b]
0.3	3600[c]	0.3 s	3 s	0.97
2.5	886[d]	1.2 s	12 s	0.999
2.9	177[d]	6.2 s	1.0 min	0.997
3.1	108[d]	10.1 s	1.7 min	0.987
3.7	62.1[d]	17.4 s	2.9 min	0.9994
4.7	23.5[d]	46.4 s	7.6 min	1
6.5	3.8[e]	4.8 min	49 min	0.9998
7.1	1.5[e]	12 min	2.0 h	0.999
7.8	0.38[e]	48 min	8.0 h	0.998

[a] Calculated for 1 % and 0.1 % (*w/v*) NCT solution (0.055 resp. 0.0055 M) after $t_{0.5} = 1/k_r[NCT]$ [17];
[b] correlation coefficient (goodness of fit of linear regression: time versus 1/[NCT] plot for second order kinetics);
[c] 0.5 M $HClO_4$;
[d] 0.5 M citrate;
[e] 0.5 M phosphate.

or weakly alkaline values. It becomes important at pH 6–8 while it is very slow in a more alkaline environment. Probably it is a two stage reaction with a primary hydrolysis step forming HOCl, which is the actual agent which transfers the halogen to taurine, a reaction which increases acidity:

$$Cl_2NCH_2CH_2SO_3^- + H_2O \leftrightarrow ClHNCH_2CH_2SO_3^- + HOCl$$

$$HOCl + H_3N^+CH_2CH_2SO_3^- \leftrightarrow ClHNCH_2CH_2SO_3^- + H_3O^+$$

Because of the inductive effect of the second chlorine atom in NDCT, the hydrolytic formation of HOCl by splitting off Cl^+ seems to be plausible.

However, it was not possible to establish a kinetic pattern for comproportionation as clear as that shown for disproportionation.

Oxidation of thio compounds (sulfhydryls, thioether)

Compounds containing SH groups like cysteine are immediately oxidised, upon which disulfides (Eq. (8)) and sulfur-oxygen acids (Eq. (9)) are formed:

$$2\,R–SH + NCT \rightarrow R–SS–R + taurine + Cl^- \quad (8)$$

$$R–SH + n\ NCT + n\ H_2O \rightarrow R–SO_nH + n\ taurine + n\ Cl^- \quad (9)$$

n = 1,2,3

Plausible intermediates are unstable S-Cl compounds which react either with another thiol molecule or with water:

$$NCT + R–SH + H_2O \rightarrow taurine + [R–S–Cl] + OH^-$$

$$[R–S–Cl] \begin{cases} + R–SH \rightarrow R–SS–R + H^+ + Cl^- \\ + H_2O \rightarrow R–SOH + H^+ + Cl^- \end{cases}$$

$$R–SOH + NCT + H_2O \rightarrow R–SO_2H + taurine + Cl^-$$

$$R–SO_2H + NCT + H_2O \rightarrow R–SO_3H + taurine + Cl^-$$

Taking into account both the decrease of oxidation capacity and the amount of precipitated insoluble cystine reveals that NCT reacts with cysteine already at room temperature in both directions at a high rate with the result that at least four reaction products, disulfides, sulfenic, sulfinic, and sulfonic acids are possible with a ratio of NCT to cysteine ranging from 1 : 2 to 3 : 1. Besides the molar ratio also the mode of mixing the reactants has a great influence. Figure 6a reveals a drastic difference in the decrease of oxidation capacity depending upon whether the NCT solution was added slowly (drop by drop) to the cysteine solution or if it was done in the reverse order. The first, very fast breakdown of oxidation capacity can be attributed to Eq. (8) which correlates largely with the amount of precipitating cystine.

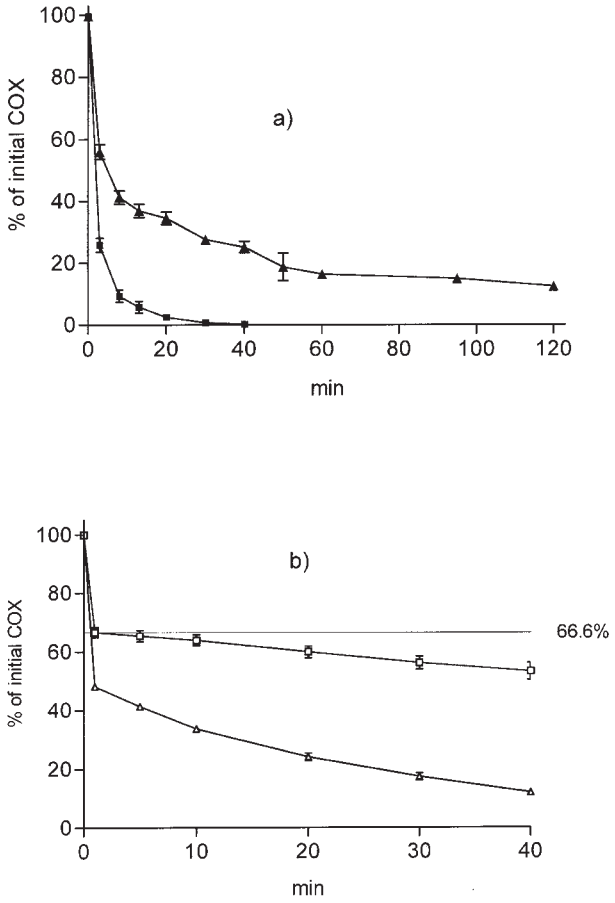


Figure 6. Reaction of NCT with thio-compounds. (a) Temporal decrease of oxidation capacity of 100 mM NCT in the presence of 50 mM cysteine at pH 7 and different modes of mixing (addition drop by drop): -▲- addition of cysteine to NCT; -■- addition of NCT to cysteine. (b) Temporal decrease of oxidation capacity of each 150 mM NCT (-□-) and CAT (-△-) in the presence of 50 mM methionine at pH 7.0 (0.2 M phosphate buffer). Each point represents the mean ± S.D. of three independent experiments; $P < 0.01$ (t test).

Thioethers like methionine predominantly interact only with one NCT molecule at pH 7 which is consistent with formation of the corresponding sulphoxide [18].

$$NCT + CH_3–S–R + H_2O \rightarrow \text{taurine} + CH_3–SO–R + Cl^- \quad (10)$$

$R = CH_2CH(NH_2)COOH$

The immediate decrease of oxidation capacity shown in Figure 6b uncovers a stoichiometric transformation with NCT according to Eq. (10). For chloramine T (CAT) which was used for comparison as a more reactive oxidant, this stoichiometric feature is not shown and the oxidation might go towards a sulfone. There follows a second reaction with a substantially slower rate for both agents which can be attributed to the typical decomposition of *N*-chloroaminocarbonic acids (see "Discussion" and Figure 7d).

Chlorination of aromatic C-H compounds

Phenol, which is known to be susceptible to substitution by halogens, reacts with NCT very slowly (Figure 7a). The reaction is accompanied with the formation of the deep blue color of indophenol created by the secondary decomposition product NH_2Cl (emerging at the reaction of NH_3 with NCT, see Eqs. (3), (15), and (11)) which combines with phenol (Berthelot reaction) [19]. CAT reacts much faster under the same conditions, however, without producing the blue colour of the Berthelot reaction.

Another aromatic compound susceptible to halogenation, the amino acid histidine, reacts very fast with NCT and with a less pronounced difference to CAT (Figure 7 b).

Chlorination of N-H compounds (transhalogenation)

The first reference to transhalogenation was from Lin et al. [8] who proved the chlorine transfer from NCT to serine. In contrast to more reactive oxidants like hypochlorite, NCT equilibrates to an appreciable amount only with

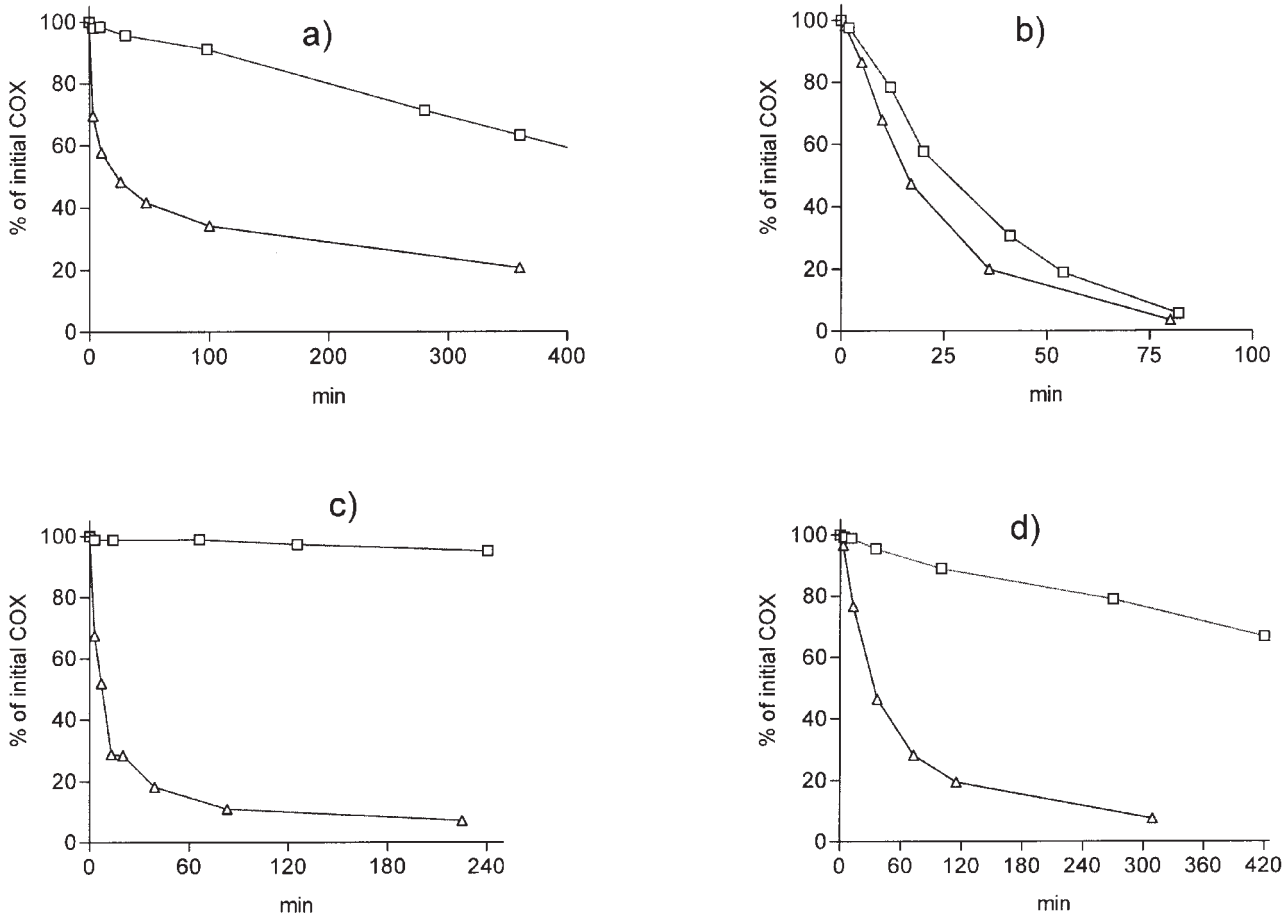


Figure 7. Reaction of NCT (-□-) and CAT (-△-) with phenol (a), histidine (b), ammonium chloride (c), and glycine (d). All reactants equivalent to 1 % (*w/v*) NCT (55 mM), pH 7 (0.05–0.1 M phosphate buffer). Each point represents a single measurement. The difference between NCT and CAT was significant ($P < 0.01$ for a, c, and d; $P = 0.0104$ for b; *t* test).

NH_3, organic amines and amino acids, but not with amides and imides.

NH_2Cl-Formation: Contrary to the *N*-chloroamino acids which are characterised by nearly the same UV absorption peak at ≈250 nm, the corresponding peak of NH_2Cl at 242 nm [16] diverges from NCT (λ_{max} = 250.9 nm) enabling a photometric assessment of the equilibration with NH_4^+:

$$HClN–CH_2–CH_2–SO_3^- + NH_4^+ \leftrightarrow NH_2Cl + H_3N^+–CH_2–CH_2–SO_3^- \quad (11)$$

$$NCT + NH_4^+ \leftrightarrow NH_2Cl + \text{taurine}$$

The equilibrium constant for this halogen transfer comes at 25 °C to

$$K_{NH2Cl} = [NH_2Cl][\text{taurine}]/[NCT][NH_4^+]\, f_a^2 = 0.02 \pm 0.004 \quad (12)$$

The NH_2Cl equilibrium concentrations calculated by Eq. (12) disclose that 0.1 % (1.0 %, 3.0 %) NCT produces 1.1 mM (3.1 mM, 4.7 mM) NH_2Cl in the presence of 0.1 % (18.7 mM) ammonium chloride. The according values were 2.4 mM (8.7 mM, 14.7 mM) NH2Cl for 1.0 % (187 mM) ammonium chloride. Upon this reaction, there is virtually no loss of oxidation capacity in the presence of NCT, which is contrary to CAT (Figure 7 c).

Reaction with glycine: There is a significant decrease of oxidation capacity in the presence of glycine (Figure 7 d), indicating an equilibration to the unstable *N*-chloroglycine:

$$NCT + H_2N–CH_2–COOH + H^+ \rightarrow \text{taurine} + ClHN–CH_2–COOH$$

The significantly faster decrease in the presence of CAT points to a considerably increased degree of conversion to *N*-chloroglycine.

Discussion

The unique status of *N*-chlorotaurine

It is surprising that NCT-Na represents until now the sole *N*-chloroamino acid which has been isolated as a pure compound. Its outstanding stability compared to other *N*-chloroamino acids can be quoted as a main explanation for this feature. However, also the impossibility of preparing the sodium salt of *N*-chloro-β-alanine (Gottardi, unpublished) is difficult to understand all the more the compound exhibits a stability in aqueous solution which suggests its isolation is feasible [20].

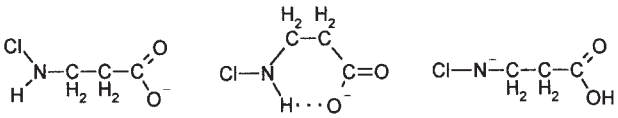
As reasons for these discrepancies can be quoted items of structure, (i) the position of the amino function (α- or β-amino acid), and (ii) the nature of the acid function (carbonic or sulfonic acid).

(i) Isolation of the *N*-chloro derivatives of α-aminocarbonic acids (which represent the majority of biologically important amino acids) has not been possible until now because of their inclination to decompose[d]. A plausible explanation for the divergence in stability between the α- and β-amino acids has its roots in the initial step of disintegration of *N*-chloroamino acids which concerns elimination of HCl leading to an unstable imine as shown in Eq. (13) for an *N*-chloro-α-amino acid [20].

(13)

Based on the inductive effect of the carboxylic group, the elimination is favoured for the NHCl function in α-position, while in a β-amino acid it is weakened by the intervening CH_2 group.

(ii) The fact that the *N*-chloro derivative of the β-amino acid taurine is easily accessible, while it is not possible with β-alanine – all attempts of its synthesis resulted in non-oxidising and non-definable material – is more difficult to understand. Molecular models of NCT-Na and *N*-chloro-β-alanine sodium do not explain this divergence. The key, however, might be the difference in acidity of the –COOH and the $–SO_3H$ groups which comes to nearly six powers of ten in the case of their methyl derivatives (CH_3COOH: pK_a = 4.75; CH_3SO_3H: pK_a = –1.20 [22]).



A B C

Therefore, it seems plausible that the sodium salt of *N*-chloro-β-alanine does not only exist in the form A – which dominates in NCT-Na according to the IR spectrum – but also in the tautomeric form C which can be formed by a rearrangement via the cyclic transition state B.

By detaching chloride from structure C (formation of NaCl by an intramolecular redox reaction) a *nitrene* would remain whose trend to undergo transmutation is known. The non-oxidising products emerging from the reaction of *CAT* with β-alanine as well as the evidence of chloride therein at least support this explanation.

Based on these considerations, the outstanding stability of NCT can be reduced to its structure as a β-aminosulfonic acid. However, this assertion concerns only its salts because the free acid does not exist on grounds of rapid disproportionation.

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[d] This assertion concerns only the free *N*-chloroamino acids, while the *N*-chloro derivatives of diverse esters of α-aminoisobutyric acid turned out to be sufficiently stable to be synthesised [21].

Another feature illustrating the unique status of NCT concerns the feasibility of its synthesis, which was not possible for *N*-chloro-γ-aminopropanesulfonic acid, the homologue of NCT (Gottardi, unpublished). Obviously, the successful and probably simultaneously occurring exchange of sodium and chlorine for two hydrogen atoms during the one-stage synthesis of NCT-Na (Eq. (14)) from chloramine-T and taurine in ethanolic solution needs a special structural condition which is not present in γ-aminopropanesulfonic acid.

$$CH_3–C_6H_5–SO_2NClNa + H_3N^+C_2H_4SO_3^- \rightarrow NCT–Na + CH_3–C_6H_5–SO_2NH_2 \quad (14)$$

Routes of decomposition of NCT

– Aqueous solution: There are two categories of reactions occurring in aqueous solution, with retention of and with loss of oxidation capacity. To the first belong hydrolysis (Eq. (2)) and disproportionation (Eq. (4)), to the latter spontaneous decomposition launched by elimination of HCl forming an imine and subsequent hydrolysis to an aldehyde and ammonia. In case of NCT decomposition to sulphoacetaldehyde (Eq. (3) and Eq. (15)) has already been proved to be a possible route for mammalian taurine metabolism [23].

$$HN=CH–CH_2–SO_3^- + H_2O \rightarrow O=CH–CH_2–SO_3^- + NH_3 \quad (15)$$

The iodometric assessment of stability of NCT-Na (Figure 1 b) complies with such an elimination because aldehydes do not oxidise iodide. Furthermore, the very sensitive Berthelot reaction (see above) originating from a cascade including Eq. (3), Eq. (15), and Eq. (11) may serve as an evidence for the development of NH_3.

– Solid NCT-Na: A somewhat differing decomposition mechanism can be discussed, probably a disproportionation in the solid state according to Eq. (16) (with the sodium salts of NDCT and taurine as intermediates) which precedes the formation of cyanomethanesulfonic acid by elimination of two molecules of HCl from NDCT-Na (see the overall reaction Eq. (1)).

$$2\ ClHNCH_2CH_2SO_3Na \rightarrow Cl_2NCH_2CH_2SO_3Na + H_2NCH_2CH_2SO_3Na \quad (16)$$

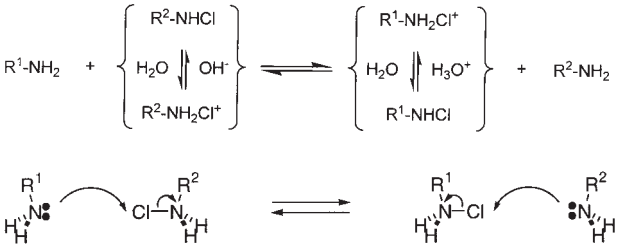
Transhalogenation

Because N–Cl bonds are easily formed and split, the equilibrium (Eq. (17)) is established already at room temperature with considerable speed.

$$R_1–NHCl + R_2–NH_2 \rightarrow R_1–NH_2 + R_2–NHCl \quad (17)$$

For this halogen transfer between two amine compounds which is characterised by retention of oxidation capacity, the term “transhalogenation” has been introduced [24] to distinguish it from other halogenations, e. g. of C–H and S–H compounds, where oxidation capacity is lost.

A mechanistic approach could imply a nucleophilic attack of the free electron pair of the amino group of the acceptor molecule R_1-NH_2 towards the chlorine atom of the protonated donor molecule $R_2–NH_2Cl^+$:



The facility of transhalogenations seems to be fundamental for the operation of the immune system. During an oxidative burst numerous reactions take place when oxidation capacity in the form of HOCl is produced which is absorbed immediately by amino compounds present in the intra- and extracellular domain. NCT, because of its superior stability and the high concentration of taurine, is an important partner for transhalogenation reactions.

A special case of transhalogenation concerns disproportionation at pH < 8 which causes the presence of taurine and NDCT at concentrations varying with pH and the time elapsed since dissolution.

NCT as a weak oxidant

The presented reactions reveal NCT to be an agent which is much less oxidising than CAT. This difference was also observed in a study on the consumption of both oxidants by organic material like human plasma and fetal calf serum [25]. Based on another publication [26] which deals with the interaction of peptone with a series of halogen compounds (including several *N*-chloro and *N*-bromo compounds as well as iodine, bromine and hypochlorite), the following ranking of active chlorine compounds has been established: hypochlorite > trichloroisocyanuric acid > 1,3-dichlorohydantoin > chloramine T. According to all of these results, NCT represents the weakest oxidising active chlorine compound accessible for use in practice.

N-chlorotaurine sodium – a novel disinfectant in human medicine

The unique stability and low reactivity of NCT prove to be the requirements for its function in the mammalian de-

fence system as a long-lived oxidant [1]. Moreover, these features are fundamental for the optimal compromise between microbicidal activity and low toxicity demonstrated in previous studies concerning the *in vitro* bactericidal [5, 9, 24], fungicidal [27], and virucidal activity [28], as well as its outstanding tolerability as an antiseptic [29–31]. Not least because of its availability in the form of the sodium salt NCT can be noted as a promising new disinfectant in human medicine.

Conclusions

Using the pure crystalline sodium salt we were able to elucidate the behaviour of NCT, both apart from and in the presence of reactants. In the first case we could (a) ascribe the outstanding stability to its structure as a β-aminosulfonic acid and (b) attribute a weakly alkaline environment necessary to keep an NCT solution free from the reaction products of disproportionation. The main feature concerning reactions might be the ease of transhalogenation which enables an equilibration with proteinaceous N–H compounds already at room temperature, a feature important in view of the role of *N*-chloro compounds in the human defence system. The reactions established for NCT can be transposed to the bulk of *N*-chloro-α-amino acids emerging (besides NCT) during the oxidative burst which, however, can not be investigated in substance because of their instability.

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Experimental part

Chemicals

N-Chlorotaurine sodium (NCT-Na) was purchased from Gatt-Koller GmbH, 6067 Absam, Austria. *N*-Chloro-4-toluenesulfonamide-sodium (CAT) and buffers were from Merck, while the amino acids were from Sigma. All reagents were of the highest available purity. All concentrations in percent are weight per volume (*w/v*).

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.

Photometric analysis of aqueous NCT, NDCT, and mixtures thereof

The absorptions at 302.4 nm (λ_{max} of NDCT) and 250.9 nm (λ_{max} of NCT) measured with a 1-cm cuvette were:

$E_{302.4} = E_2 = [NCT] \times \varepsilon_1 + [NDCT] \times \varepsilon_2$

$E_{250.9} = E_1 = [NCT] \times \varepsilon_3 + [NDCT] \times \varepsilon_4$

which can be transformed to:

$[NCT] = (E_1 - \varepsilon_4 \times E_2/\varepsilon_2)/(\varepsilon_3 - \varepsilon_1 \times \varepsilon_4/\varepsilon_2)$

$[NDCT] = (E_2 - \varepsilon_1 \times E_1/\varepsilon_3)/(\varepsilon_2 - \varepsilon_1 \times \varepsilon_4/\varepsilon_3)$

The molar absorption coefficients were measured with a Lambda 20 UV/VIS Photometer from Perkin Elmer. To avoid any disproportionation, NCT-Na was dissolved in aqueous 0.01 M Na_2HPO_4 solution which was adjusted with NaOH to pH 9.7. Since taurine shows no absorption at >250 nm, a NCT-Na sample completely disproportionated in 2N H_2SO_4 could be used as a NDCT standard.

The concentration of the NCT and NDCT solution was verified by iodometric titration.

302.4 nm: $\varepsilon_{NCT} = \varepsilon_1 = 22.4$ L mol^{-1} cm^{-1},

$\varepsilon_{NDCT} = \varepsilon_2 = 332.9$ L mol^{-1} cm^{-1}

250.9 nm: $\varepsilon_{NCT} = \varepsilon_3 = 397.4$ L mol^{-1} cm^{-1},

$\varepsilon_{NDCT} = \varepsilon_4 = 220.1$ L mol^{-1} cm^{-1}

Estimation of pK_b

From Eq. (2) follow the mass balances

$c(NCT) = [NCTH^+] + [NCT] = C$

and

$[OH^-] = [NCTH^+]$

which allow the definition

$pK_b = [NCTH^+]\ aOH^-/[NCT] = [OH^-]^2/(C\text{-}[OH^-])$

$\approx KW^2/C(aH^+)^2 f_a^2$

The course of pH was scanned for ten min when weighed samples of NCT-Na (50–200 mg) were dissolved at zero time in 10.0 mL portions of stirred distilled water at 25.0 °C. The pH at zero time was estimated by graphic extrapolation.

The pK_b was 7.9 ± 0.2 (mean value ± standard deviation, $N = 19$).

Evaluation of the disproportionation constant

From Eq. (4) follows [NDCT] = [taurine], and Eq. (5) can be rearranged to

$K_d = [NDCT]^2/f_a[NCT]^2 aH^+$.

A solution of 0.0058 mol/L NCT-Na (0.63 g in 600 mL) containing 0.05 M phosphate buffer was divided in 6 parts which were adjusted to initial pH between 4.0 and 7.4 and kept in the dark at room temperature. Using the UV spectrum the progress of disproportionation (see above) was monitored for several days until no further change could be observed. From the final pH values between 5.24 and 7.45 and the photometrically assessed equilibrium concentrations of NCT and NDCT the disproportionation constant was found to be $K_d = 4.5 \pm 0.8 \times 10^6$; $N = 6$

Kinetic measurements

The pH dependence of disproportionation was investigated in the pH range of 0.3–7.8 using 0.5 M of $HClO_4$ (pH 0.3), citrate buffer (pH 2.5–4.7), or phosphate buffer (pH 6.5–7.8). One

compartment of a double chamber cell (each 0.437 cm) was filled with 1 mL of a NCT-Na solution (6.0–9.0 × 10^{-3} M) in water and the other with 1 mL of the 1 M buffer solution. After 10 min at 25.0 °C in the thermostatted cell holder the first scan was made. It was used for the calculation of the [NCT] at $t = 0$. Then the contents of both compartments were mixed and scanned at appropriate times (max 60 min). The NCT concentrations in the resulting NCT/NDCT mixtures were calculated as described above. Since the plots of $1/[NCT]_i$ against t_i were linear for the different pH values, the disproportionation reaction is of second order with slopes indicating the observed rate constants (Table 1).

Statistics

Student's paired *t* test as well as one way analysis of variance (ANOVA) and Dunnett's Multiple Comparison test (Graphpad Software Inc., CA, USA) were applied. *P* values < 0.05 were considered significant.

References

[1] M. B. Grisham, M. M. Jefferson, D. F. Melton, E. L. Thomas, *J. Biol. Chem.* **1984**, *259*, 10404–10413.

[2] E. L. Thomas, *Infect. Immun.* **1979**, *23*, 522–531.

[3] K. Fukuda, Y. Hirai, H. Yoshida, T. Nakijima, T. Usui, *Clin. Chem.* **1982**, *28*, 1758–1761.

[4] P. Soupart in *Amino acid pools* (Ed.: J. T. Holden), Elsevier, Amsterdam, **1962**, pp. 220–262.

[5] M. Nagl, M. Hess, K. Pfaller, P. Hengster, W. Gottardi, *Antimicrob. Agents Chemother.* **2000**, *44*, 2507–2513.

[6] J. Marcinkiewicz, *Immunol. Today* **1997**, *18*, 577–580.

[7] T. Stelmaszynska, J. M. Zgliczynski, *Eur. J. Biochem.* **1974**, *45*, 305–312.

[8] Y. Y. Lin, C. E. Wright, M. Zagorski, K. Nakanishi, *Biochim. Biophys. Acta* **1988**, *969*, 242–248.

[9] M. Nagl, W. Gottardi, *Hyg. Med.* **1992**, *17*, 431–439.

[10] J. H. Fellman, *J. Labelled Compd. Radiopharm.* **1981**, *18*, 765–768.

[11] J. M. Antelo, F. Arce, P. Calvo, J. Crugeiras, A. Rios, *J. Chem. Soc., Perkin Trans.* **2000**, *2*, 2109–2114.

[12] A. M. Cantin, *J. Clin. Investig.* **1994**, *93*, 606–614.

[13] E. L. Thomas, M. B. Grisham, M. M. Jefferson, *Methods Enzymol.* **1986**, *132*, 569–585.

[14] J. M. Zgliczynski, T. Stelmaszynska, J. Domanski, W. Ostrowski, *Biochim. Biophys. Acta* **1971**, *235*, 419–425.

[15] A. C. Carr, C. L. Hawkins, S. R. Thomas, R. Stocker, B. Frei, *Free Radical Biology & Medicine* **2000**, *30*, 526–536.

[16] M. P. Snyder, D. W. Margerum, *Inorg. Chem.* **1982**, *21*, 2545–2550.

[17] H. E. Avery, *Basic Reaction Kinetics and Mechanisms,* The Macmillan Press Ltd., London, **1974**, Chapter 2 and 3.

[18] D. S. Mahadevappa, S. Ananda, N. M. M. Gowda, K. S. Rangappa, *J. Chem. Soc. Perkin Trans. II* **1985**, 39–43.

[19] Ch. J. Patton, S. R. Crouch, *Anal. Chem.* **1977**, *49(3)*, 464–469.

[20] W. Gottardi, V. Bock in *Fourth conference on Progress in Chemical Disinfection* (Ed: G. Janauer), University of Binghamton (N.Y.), **1988**, p. 35–60.

[21] J. J. Kaminski, M. M. Huycke, S. H. Selk, N. Bodor, T. Higuchi, *J. Pharm. Sci.* **1976**, *65*, 1737–1742.

[22] A. Albert, E. P. Serjeant, *The determination of ionization constants* Chapman and Hall Ltd, London, **1971**, Chapter 8.

[23] C. Cunningham, K. F. Tipton, H.B. Dixon, *Biochem. J.* **1998**, *330* , 939–945.

[24] M. Nagl, W. Gottardi, *Hyg. Med.* **1996**, *21*, 597–605.

[25] W. Gottardi, M. Hagleitner, M. Nagl, *J. Pharm. Pharmacol.* **2001**, *53*, 689–697.

[26] W. Gottardi, *Zbl. Bakt. Hyg. I. Abt. Orig. B.* **1976**, *162*, 384–388.

[27] M. Nagl, C. Lass-Floerl, A. Neher, A. R. Gunkel, W. Gottardi, *J. Antimicrob. Chemother.* **2001**, *47*, 871–874.

[28] M. Nagl, C. Larcher, W. Gottardi, *Antiviral Res.* **1998**, *38*, 25–30.

[29] M. Nagl, B. Teuchner, E. Pöttinger, H. Ulmer, W. Gottardi, *Ophthalmologica* **2000**, *214*, 111–114.

[30] M. Nagl, B. Miller, F. Daxecker, H. Ulmer, W. Gottardi, *J. Ocular Pharmacol. Ther.* **1998**, *14*, 283–290.

[31] M. Nagl, B. Pfausler, E. Schmutzhard, M. Fille, W. Gottardi, *Zent. bl. Bakteriol.* **1998**, *288*, 217–223.