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ORIGINAL RESEARCH PAPER

Fate of tris(2-chloroethyl)amine in water and alkaline environment determined by thin-layer chromatography and gas chromatography–mass spectrometry

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Abstract
Tris(2-chloroethyl)amine is a chemical warfare agent which is considered to be a persistent contaminant highly resistant to decontamination. The time dependence of tris(2-chloroethyl)amine degradation on the water pH value is observed to determine decontamination options using the decontamination of hydroxide-based mixtures which are used by a number of armed forces. The kinetics was observed using thin-layer chromatography and gas chromatography. The time development of tris(2-chloroethyl)amine concentration decrease in the environment with the pH ranging from 7.5 to 12.5 was recorded. The reaction rate constants were established at all observed pH values, half-lifes of reactions and the influence of temperature on the reaction rate at a slightly alkaline pH of 8.5. The equation to estimate the rate constant of the first step of tris(2-chloroethyl)amine hydrolysis at various temperatures was formed. The rate of triethanolamine formation in the samples of neutral to alkaline pH was recorded. The results indicate the substantial influence of pH on tris(2-chloroethyl) amine hydrolysis. The rate constant was significantly increased from a neutral environment to the pH of 12.5 reaching 500% of the original value using alkalisation. The hydrolysis in a neutral environment was also influenced by the analyte concentration. The concentrated solutions hydrolysed more slowly as a result of hydrochloric acid release as the acid lowered the pH value of the environment. The results demonstrate that there is an important kinetic difference between sulphur and nitrogen mustard hydrolysis in an alkaline environment.

Keywords Nitrogen mustard · Hydrolysis · Reaction rate constant · Arrhenius equation · Decontamination

1 Introduction

Tris(2-chloroethyl)amine is a vesicant, also called nitrogen mustard HN-3. After the first synthesis [1], a potential war deployment [2] was studied by several armed forces. Concerning the discovery of agent alkylating effects, HN-3 was used as a chemotherapeutic agent whilst curing lymphoma or leukaemia [3, 4]. HN-3 is stable in an acidic environment depending on the form (base, hydrochloride), and it is soluble in water as well as fat and easily synthesised. It can be considered as a potentially abused substance to contaminate water and groceries by terrorists. Concerning less developed states, HN-3 can be considered as a "quick and dirty option" when considering the chemical warfare agents production. The substance can be found in Schedule 1A of the Chemical Weapons Convention [5].

Several authors [6–8] mention considerable resistance of HN-3 against hydrolysis. The combination of oxidation and chlorination reactions has been recommended for decontamination. Franke [8] explicitly mentions the unsuitability of alkaline hydrolysis usage for the HN-3 decontamination in field conditions due to the low reaction rate. The environmental fate of the substance when militarily deployed can be also an issue.

In a polar environment, the induction effect intensification of chlorine atom effect occurs and subsequently, *N*,*N*-bis(2-chlorethyl)aziridinium chloride is formed in isomerisation. The isomer of HN-3 molecule in the presence of a nucleophilic agent undergoes the substitution of chloride ion. It is a monomolecular nucleophilic substitution S_N1 when the formation of aziridinium ion is the slowest process. The fact

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is probably the source of the statement that alkaline substances have a low influence on hydrolysis acceleration. The hydrolysis occurs in three steps. Isomerically formed *N*,*N*-bis(2-chlorethyl)aziridinium chloride can act as an intermediate of the cascade of substitution reactions [9]. The main products are *N*,*N*-bis(2-chloroethyl)-*N*-2-hydroxyethylamine (HN-3-OH) and its salt, respectively. The subsequent substitution of the residual atoms of chlorine, again whilst forming cation intermediates (Fig. 1), occurs in the following steps.

Nitrogen mustard hydrolytic products are usually determined using high-performance liquid chromatography/mass spectrometry (HPLC/MS) [10–17] and gas chromatography/mass spectrometry (GC/MS) [18–23]. However, the data connected to the kinetics of HN-3 hydrolysis are difficult to obtain. *Golumbic et al*. [24] studied HN-3 hydrolysis in a non-buffered water and in a buffer solution at the pH of 8. It was observed that with the sample in non-buffered environment, the equimolar amount of HCl is released after 20 h and HN-3-OH hydrochloride is the product of the reaction. *N*-2-chloroethyl-*N*,*N*-bis(2-hydroxyethyl)amine (HN-3-$(OH)_2$) hydrochloride and triethanolamine (TEA) as a minor component were isolated after 72 h. HN-3 isomerisation to *N*,*N*-bis(2-chloroethyl)aziridinium chloride occurred at the pH of 8 after 15 min, whilst *N*-2-chlorethyl-*N*-2-hydroxyethylaziridinium chloride was created after 60 min. 95% of the mixture consisted of TEA after 20 h. The authors mention that with the gradual substitution of chlorine atoms, HN-3 molecule is less disposed to isomerisation and the further steps of hydrolysis are considerably slower. *Sartori* [9] states that HN-3 water solution consists of the mixture of degradation products with 4% content of piperazinium salt after 72 h. There is also a statement that after 24 h, spontaneous hydrolysis of HN-3 occurs in 50% after 24 h [25]. The commonly stated final product—TEA—also undergoes dimerisation whilst forming 4-morpholine ethanol according to the authors.

Lee et al. [26] state that at the pH of 7, HN-3 was degraded after 72 h. It is also proven that HN-3, unlike HN-1 and HN-2, does not have the tendency to form piperazinium salt in diluted solutions. It was also proven that the concentrated samples have a higher tendency to undergo dimerisation in comparison with hydrolysis. Literature does not offer the experimentally ascertained kinetics parameters of HN-3 hydrolysis which would support the mentioned theory claiming the unsuitability to use decontamination mixtures based on hydroxides for decontamination.

The objective of the study is to verify the influence of pH on the degradation of HN-3 using the technology of thin-layer chromatography (TLC) and GC/MS and to determine the basic kinetic data of hydrolysis of the chemical warfare agent. Another objective is to demonstrate the potential to

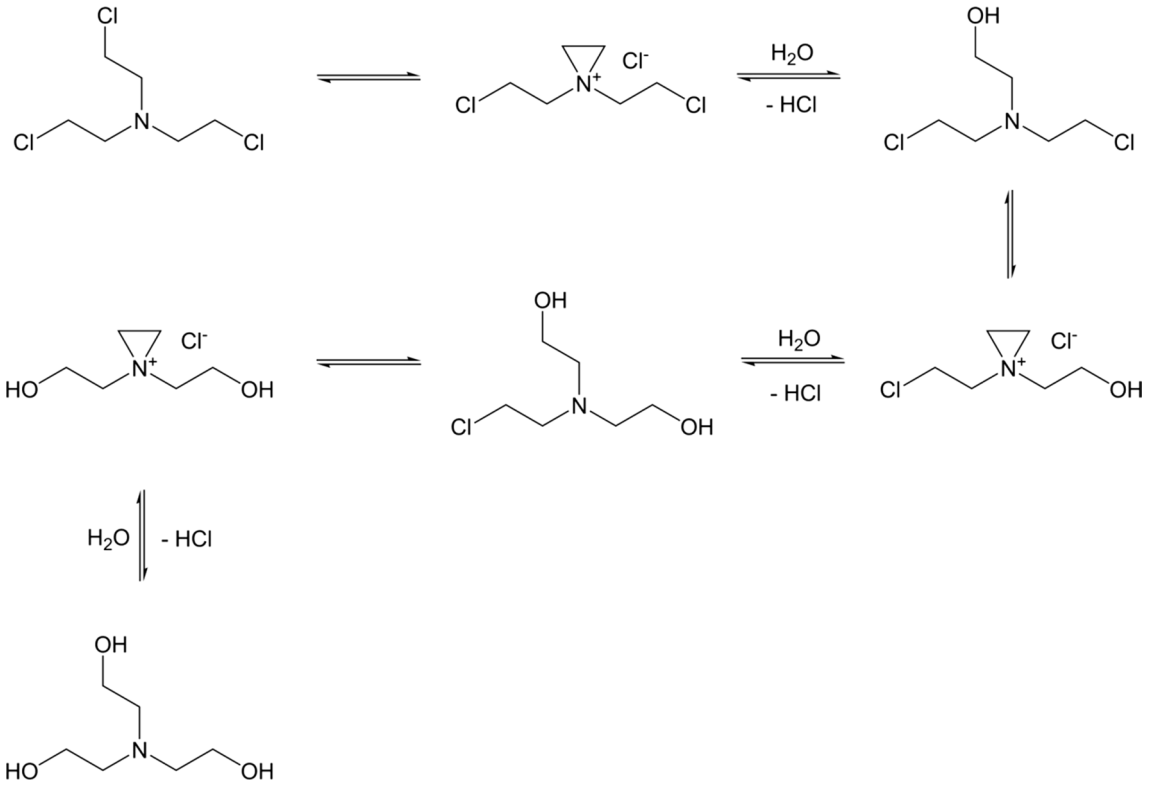

Fig. 1 Tris(2-chloroethyl)amine hydrolysis scheme

use TLC as a safe and simple technology to study the kinetics of the reactions of toxic substances.

2 Experimental

Tris(2-chloroethyl)amine hydrochloride (HN-3·HCl) was produced in VOZ Zemianské Kostoľany (Slovakia). The purity (99%) was determined using GC/MS and the melting point (128.9 ± 0.56 °C) using Melting Point M-565 (Büchi, Switzerland). The buffer solutions (pH 7.5–12.5) were based on the mixtures of boric acid p.a. (Lachema, Brno, Czech Republic) ($pK_a = 9.4$) with hydrochloric acid 35% or sodium hydroxide p.a. (both PENTA, Prague, Czech Republic) according to the literature [27] and checked using pH meter HI-213 with a glass bodied ion-selective electrode HI 1131 B (HANNA Instruments, Woonsocket, RI, USA).

Benzene, Chromapur G (Chromservis, Praha-Petrovice, Czech Republic), methanol 99.9%, triethylamine 99% (both Sigma-Aldrich, Steinheim,Germany), potassium permanganate (Lachema), phosphoric acid 85% (PENTA) and triethanolamine p.a. (Lachner, Czech Republic) were used in TLC. Dichloromethane p.a. (Merck, Darmstadt, Germany), tributyl phosphate 99% and magnesium sulphate 97% (both Sigma-Aldrich) were used in GC.

TLC was conducted using a twin-trough developing chamber (CAMAG, Muttenz, Switzerland), chromatographic plates TLC silica gel 60 F_{254} (Merck), disposable micropipettes 1–5 µL, ventilated TLC Spray Cabinet II (all CAMAG) and sprayer SG 1 (DESAGA, Germany).

GC/MS was conducted using mobile GC/MS system EM 640 (electron ionisation, quadrupole mass filter, *m/z* 50–550, splitless injection). For the acquisition and evaluation of the data, software packages m.a.c.s. LabStar and Bruker DataAnalysis (all Bruker, Mannheim, Germany) were used. Microlitre syringes (Hamilton, Reno, NV, USA) were used for injection. The extraction was conducted using the shaker IKA KS 125 basic (IKA Labortechnik, Staufen im Breisgau, Germany). Thermostat HD-4 (Julabo, Seelbach, Germany) was used to control the temperature of the water phase.

3 Procedures

3.1 TLC

The developing chamber with the dimensions of 13 × 13.5 cm with a filtration paper with the dimensions of 10 × 10 cm inside was used for the screening of suitable mobile phases. The mobile phases were prepared by mixing volume ratios of separate solvents and placed into the chamber in the amount of 10 mL. The mixtures of benzene−methanol, dichloromethane−methanol, *n*-butanol−pyridine, and ethyl acetate−methanol were tested as well as the influence of using ammonia or triethylamine as admixtures. Before inserting TLC plates, the chambers were saturated with vapours for a period of 30 min at the laboratory temperature. The chromatographic plates saturated with a fluorescent indicator with the dimensions of 5 × 10 and 10 × 10 cm (activated by drying at the temperature of 110 °C and stored in the desiccator) were used. Whilst screening the mobile phases, the solutions with the volume of 10 µL with the concentrations of 2 mg mL^{-1} (8,3 mmol dm^{-3}) HN-3·HCl (solution 1), 1,25 mg mL^{-1} (8,3 mmol dm^{-3}) TEA (solution 2), and 2 mg mL^{-1} HN-3·HCl after 30 min in the buffer of the pH of 8.5 (solution 3) were applied at the start line of the plate. The plates were conditioned in the chamber for 20 min before having been developed. The retardation factors of the analytes, the separation efficiency and the stains tailing were observed. The solution of 2 mg mL^{-1} of $KMnO_4$ with the admixture 4 mL of H_3PO_4 was used as the derivatisation reagent. $KMnO_4$ converts tertiary amines to amine oxides which can be observed as white stains on the purple background on the TLC plate.

2–10 µL (with the increase of 2 µL) of the solution of 2 mg ml^{-1} of HN-3·HCl in methanol was applied on the plates to form the calibration dependence. The same procedure was abode when using 1.24 mg ml^{-1} of TEA in water. Benzene−methanol−triethylamine (8.5:1.5:0.02, *V/V*) mixture was used as the mobile phase. The plate was left free for a period of 30 min after application of the derivatisation reagent and then, the size of stains was measured using a sliding measuring gauge. Each calibration range was measured five times.

Subsequently, the time dependence of HN-3 degradation in the buffer environment with the pH ranging from 7.5 up to 12.5 was measured. The solution of 2 mg ml^{-1} of HN-3·HCl in the given buffer was created, and it was preserved at the temperature of 25 °C. The amount of 20 µL was removed in sequential intervals and applied to the plates. Each plate was developed after the application of the hydrolysed mixture and derivatised by the optimised procedure. The development of the stain sizes of the samples sampled at different intervals was observed. Each experiment was performed five times, and the arithmetic means and corresponding deviations were calculated.

3.2 GC/MS

The residual amount of HN-3 in the buffer environment was also measured using GC/MS. The solutions of 0.1, 0.5, and 1.0 mg mL^{-1} of HN-3·HCl in water with the volume of 3 mL were formed in order to ascertain the calibration dependence of the instrument response. 3 mL of dichloromethane spiked by tributyl phosphate (200 nL mL^{-1}) were added to the solutions. The extraction was conducted by 5 min shaking on the

shaker at the rate of 800 rpm. Subsequently, 2 µL of dichloromethane extract was manually injected into the injection port of GC. The ratio between the peak areas of HN-3 and tributyl phosphate was measured.

The buffers with the pH ranging from 7.5 up to 12.5 were formed. HN-3·HCl was always dissolved in the concentration of 1 mg mL^{-1} in the buffer of the corresponding pH value and preserved at the temperature of 25 °C. After the ascertained period, the extraction into dichloromethane spiked by tributyl phosphate was conducted. Afterwards, the extract was injected into the injection port of GC. The samples were measured at the various stages of HN-3 hydrolysis in the buffer up to the period when the analyte was not detectable. Each experiment was performed five times and the arithmetic means and corresponding deviations were calculated.

The influence of higher temperature on HN-3 hydrolysis was also measured. The results measured at the pH of 8.5 were compared. It was proceeded in a similar way like in the previous case, but after the dissolving of HN-3·HCl in the buffer with the pH of 8.5, the samples were placed into a water bath with the temperature of 40 °C. The sample was rapidly cooled before the extraction.

4 Results and discussion

4.1 TLC: mobile phase selection

The optimal mobile phase to separate HN-3 and its hydrolytic products using TLC was searched. HN-3 molecule is decomposed into *N*,*N*-bis(2-chloroethyl)-*N*-2-hydroxyethylamine (HN-3-OH) in the first step of hydrolysis, which is subsequently hydrolysed into *N*-2-chloroethyl-*N*,*N*-bis(2-hydroxyethyl)amine (HN-3-$(OH)_2$). In the third step of the hydrolysis, TEA is formed (Fig. 1). Concerning the lack of HN-3-OH and HN-3-$(OH)_2$ standards, only the concentration of HN-3 and TEA was determined in the mixtures; however, the stain presence of all degradation products was recorded. When searching for a suitable mobile phase, there existed an effort to discover the mixture which would be the most suitable to separate all four analytes and simultaneously would not cause stains tailing.

The tested mobile phases, their composition, and the retardation factors of the detected analytes are provided in Table 1. The troublesome factor was mainly the polarity of mobile phases and analytes mixtures. HN-3 is in the mixtures of tested buffers gradually decomposed into polar degradation products and in the case of TEA, it is a highly polar compound. When using insufficiently polar mobile phases, the low separation efficacies were recorded; furthermore, TEA did not migrate (remained at the start line). When using mobile phases with the relatively high content of polar components, the high values of the retardation factor were measured in the case of the least polar analyte (HN-3). In addition to that, the strong interactions of the analytes in the stationary phases during migration were frequently recorded, which was demonstrated as the tailing stains after the visualisation. It can be deduced that the hydrolysis continued during the development in the highly polar environment of the mobile phase. Out of the given selection, the mixture benzene−methanol−triethylamine (8.5:1.5:0.02, *V/V*) was the most suitable mobile phase. Triethylamine operated as a suitable additive to prevent tailing which occurred with HN-3-OH, HN-3-$(OH)_2$ and TEA (as a result of the interaction of base substances with mildly acidic silica gel).

Table 1 Results of tested mobile phases whilst separating tris(2-chloroethyl)amine and its hydrolytic products

Composition	Component ratio (*V/V*)	R_F (A)	R_F (B)	R_F (C)	R_F (D)	t_R (min)	Stains tailing
Dichloromethane−methanol−ammonia	90:9:1	0.70	0.57	0.23	0.05	13.0	No
	90:7:3	0.70	0.58	0.11	0.04	13.3	No
	95:4.9:0.1	0.68	0.53	0.10	0.01	12.5	No
n-Butanol−pyridine	1:1	0.78	0.50	0.39	0.31	40.0	Yes
n-Butanol−pyridine−water	50:35:15	0.78	0.29	0.28	0.49	41.7	Yes
Dichloromethane−methanol	95:5	0.63	0.46	0.13	0.00	12.7	Yes
Dichloromethane−methanol−triethylamine	95:4.8:0.2	0.65	0.49	0.07	0.00	12.2	No
	95:4.9:0.1	0.66	0.53	0.10	0.03	11.7	No
Ethyl acetate−methanol	95:5	0.65	0.49	0.06	0.00	11.5	Yes
Ethyl acetate−methanol−ammonia	95:4.9:0.1	0.61	0.48	0.13	0.01	9.8	No
Benzene−methanol−triethylamine	9:1:0.01	0.64	0.38	0.10	0.03	11.3	Yes
	9:1:0.02	0.65	0.38	0.10	0.03	11.6	Yes
	8.5:1.5:0.01	0.69	0.40	0.15	0.03	13.0	No
	8.5:1.5:0.02	0.69	0.46	0.20	0.04	13.0	No

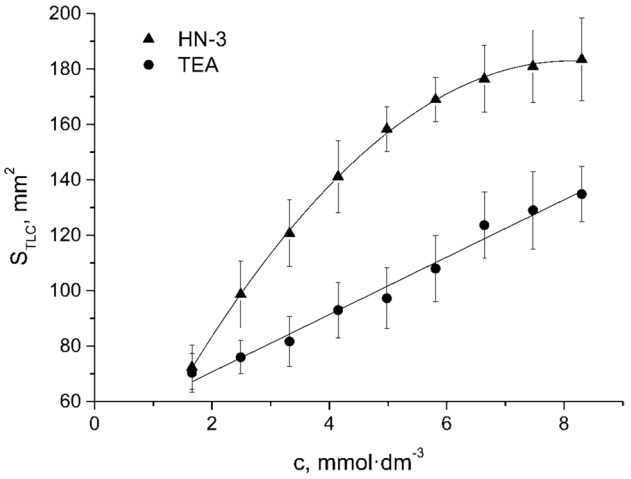

Fig. 2 Calibration dependence of visualised stains sizes on the concentration of tris(2-chloroethyl)amine (HN-3) and triethanolamine (TEA) in sample

4.2 TLC: calibration curve

The dependences of the visualised stains sizes on HN-3 and TEA concentrations in the samples were formed. The calibration curves, out of which the analyte concentration was deducted in the following experiments, are depicted in Fig. 2. Concerning TEA, the dependence was linear in the given concentration interval. The trend was interposed by the function $y = 10.361x + 49.945$. Concerning HN-3, the concave dependence was formed and it was approximated by the polynomic function $y = -2.665x^2 + 43.217x + 7.692$. The coefficients of determination dependence reached the values of 0.9995 (HN-3) and 0.9796 (TEA).

It was possible to record four stains on the plates in various time intervals during alkaline hydrolysis, whilst the stain with the highest retardation factor (0.69) corresponded with HN-3 standard and the stain with the lowest retardation factor (0.04), which appeared in the samples at the latest, corresponded with TEA standard. The stain with the retardation factor of 0.46 was formed as the first degradation product and therefore, HN-3-OH was identified. The stain with the retardation factor of 0.20 was formed as the second degradation product and therefore, it was marked HN-3-$(OH)_2$. The proposal was based on the polarity of the mentioned substances which grow in the line HN-3 → HN-3-OH → HN-3-$(OH)_2$ → TEA. When conducting TLC, the rate of HN-3 decrease in the samples during alkaline hydrolysis and the rate of TEA appearance were observed. The stains size, which determined the analyte concentration, was characterised by standard deviation, which reached up to 20%.

4.3 TLC: influence of pH on the rate of HN-3 degradation

The rate of HN-3 degradation in an alkaline environment was recorded as the concentration decrease of the original substance depending on time and it is depicted in Fig. 3. Due to clarity, the time decrease of HN-3 concentration in the environment of the pH of 7.5, where the absence of the analyte was recorded after 1500 min, is not provided in the figure. The increase of pH up to the value of 8.5 subsequently led to the significant influence of hydrolysis rate. HN-3 was not detectable in the sample just after 70 min. The further increase in pH resulted in systematic HN-3 degradation acceleration. When releasing the analyte into the environment with a pH of 12.5, HN-3 was not detectable in the sample after 20 min.

HN-3 hydrolyses by pseudo-monomolecular reactions of the first order according to the following simplified Eq. (1):

$$\mathrm{HN-3} \xrightarrow{k_1} \mathrm{HN-3-(OH)} \xrightarrow{k_2} \mathrm{HN-3-(OH)_2} \xrightarrow{k_3} \mathrm{TEA} \quad (1)$$

The rate equation for the first-order reaction allows to determine the rate constant k_1. The reaction of the first order happens exponentially, Fig. 4 depicts the linear dependence of the HN-3 concentration logarithm on the time in separate alkaline environments. The figure also provides the determination coefficients R^2 for each dependence.

4.4 TLC: influence of pH on TEA rate of appearance

The time development of TEA formation in the samples of hydrolysed HN-3 was recorded. The curves indicating the increase of analyte concentration at the separate values of

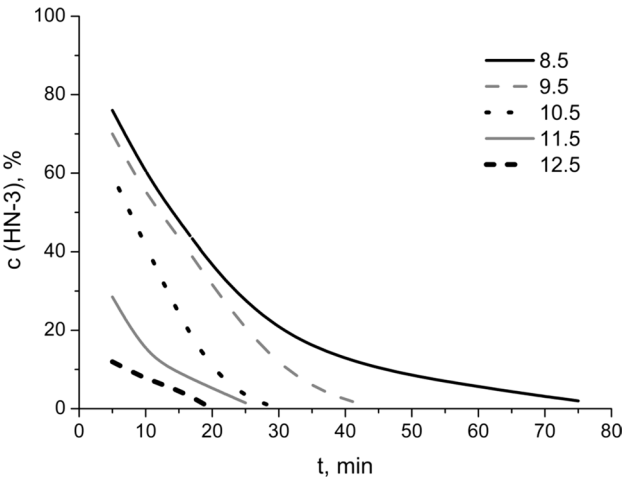

Fig. 3 Time decrease of residual tris(2-chloroethyl)amine (HN-3) concentration in solutions at various values of pH determined by thin-layer chromatography

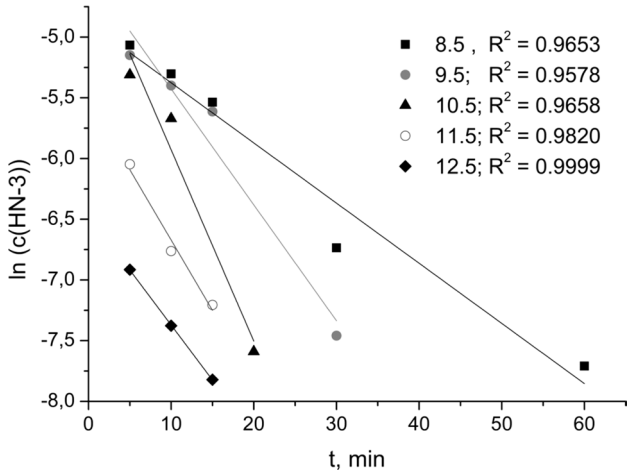

Fig. 4 Dependence of logarithmic value of residual tris(2-chloro-ethyl) amine concentration on time in separate equations with pH values of 8.5–12.5 detected using thin-layer chromatography

the pH environment are provided in Fig. 5. In the case of the hydrolysed sample of HN-3 in the buffer environment with the pH of 7.5, the maximum concentration of TEA (75%) was recorded after 2000 min and the further hydrolysis was suspended. The main reason was the significant decrease of the pH of the sample as a result of the hydrolysis of separate reactants in the sample as well as releasing the equivalent amount of the acid (Fig. 1). The value of the pH environment decreased under the values of pK_a of separate reactants, which formed hydrochlorides as a result.

In the case of the different values of pH, the third step of HN-3 hydrolysis completion (i.e. to record the presence of TEA only in the sample) in different time values was detected. In the case of the pH value of 8.5, the hydrolysis was successfully terminated after 3000 min and in the case of the pH value of 12.5, the maximum concentration of TEA was recorded only after 120 min after the beginning of hydrolysis. The phenomenon correlates with the different rates of HN-3 degradation, that is with the rates in the first step of hydrolysis. All three steps of HN-3 hydrolysis are pseudo-monomolecular reactions of the first order (Fig. 1). The curve development depicting the increase of TEA concentration is not exponential as a result of parallel development of all steps of hydrolysis mainly in the first half of the curve. Nevertheless, it is possible to deduce the period necessary for the complete transformation from HN-3 to TEA from the curve development.

4.5 GC/MS: calibration curves

To form the calibration curves, the dependence of the ratio HN-3 and tributyl phosphate (with the constant concentration in dichloromethane 200 nL mL^{-1}) peak areas on HN-3 concentration in the sample was recorded. Repeating the measurements at the beginning of each measuring day, the results with the relative standard deviation up to 10% were recorded.

4.6 GC/MS: pH influence on HN-3 degradation rate

The decrease of HN-3 concentration in time when being released into the environment with a different pH is illustrated in Fig. 6. In the case of a neutral environment in the form of borate buffer with the pH of 7.5, the neutral concentration of the analyte was recorded (or more precisely, the analyte was impossible to record using the method) after

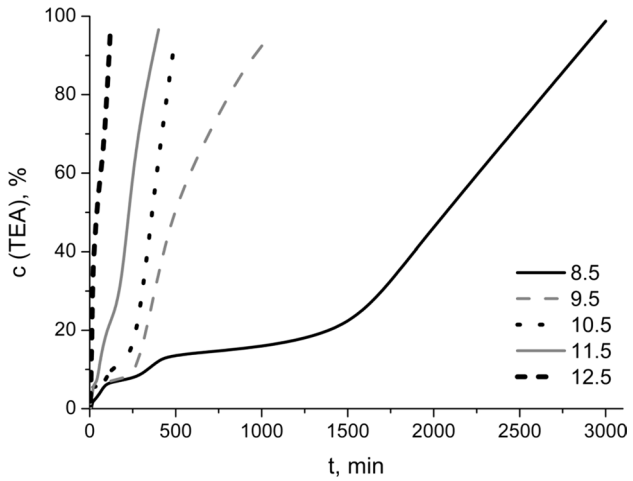

Fig. 5 Time increase of triethanolamine (TEA) concentration in solutions at various values of pH determined by thin-layer chromatography

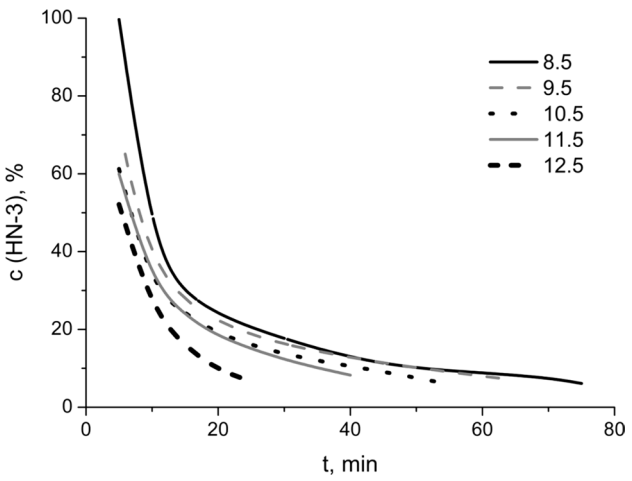

Fig. 6 Time decrease of residual concentration of tris(2-chloroethyl) amine (HN-3) in solutions with various pH values measured using gas chromatography

300 min. The increasing value of pH also caused a significant rate increase of the hydrolytic reaction. Increasing the pH value up to 8.5 caused HN-3 degradation in 80 min. In the case of the pH of 9.5, the HN-3 presence in the extract was not recorded in the hydrolysis lasting 70 min. The analyte was decomposed in 60 min in the buffer with a pH value of 10.5. When increasing pH by one unit, the period of HN-3 full degradation decreased to 45 min and with the pH of 12.5, HN-3 was not detectable in the sample after 30 min. The significant influence of pH on the rate of HN-3 degradation was apparent in the same way as when analysing using TLC.

The significant difference in the pH of 7.5 when using TLC (the solution of 2 mg mL^{-1} of HN-3·HCl) and GC/MS (the solution of 1 mg mL^{-1} of HN-3·HCl) can be observed. The significant discrepancy was not apparent with the other values of pH. To verify it, the series of the solutions of 2 mg mL^{-1} HN-3·HCl using GC/MS was tested and it was confirmed that in this concentration of the solution, HN-3 is decomposed in a slower way. The decrease of pH in the presence of more concentrated HN-3·HCl (or more precisely, releasing the higher amount of HCl when hydrolysing the more concentrated analyte) can be determined as a cause. To verify the hypothesis, the pH of HN-3·HCl solutions (with the concentrations of 1 mg mL^{-1} and 2 mg mL^{-1}) before the hydrolysis and after the period of 7 days (Table 2) was determined. The significant decrease of the pH value with the concentration of 2 mg mL^{-1} was apparent in this case. The presence of HCl in the sample results in the decrease of pH under the value of pK_a of HN-3 (4,6) and prevents the continuation of hydrolysis. With the higher values of pH, the hydrolysis was not influenced by this phenomenon.

The dependence of the logarithm of HN-3 concentration on time in the separate alkaline environments is illustrated in Fig. 7. The use of GC/MS resulted in the determination of values with the higher coefficients of determination R^2 than in the same of semi-quantitative TLC.

4.7 Determination of rate constants

The observed rate constants of the pseudo-monomolecular reactions of the first order k_1 (the first step of HN-3 hydrolysis) in alkaline environment were determined. The rate

Table 2 Comparing values of pH of buffer samples at beginning of measurement and after 7 days at different tris(2-chloroethyl)amine hydrochloride concentration in solutions

pH	2 mg mL^{-1}	1 mg mL^{-1}
7.5	2.5	5.0
8.5	7.0	7.5
9.5	9.0	9.5
10.5	10.5	10.5
11.5	11.5	11.5
12.5	12.5	12.5

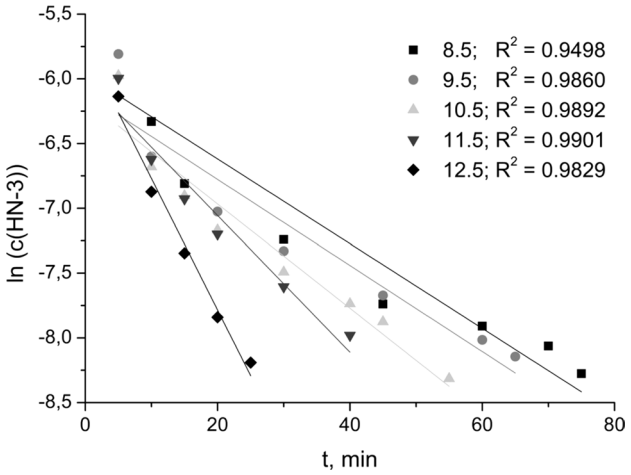

Fig. 7 Dependence of logarithm value of residual tris(2-chloroethyl) amine concentration on time in separate samples with pH values of 8.5−12.5 using gas chromatography

constants were ascertained using the integral form of the rate equation for the reactions of the first order (2):

$$c_{HN-3} = c_{HN-3(0)} \cdot e^{-(k_1 \tau)} \quad (2)$$

The rate constants determined using TLC and GC/MS are stated in Table 3. Concerning the significant deviations when determining concentration using TLC, the values of the rate coefficient ascertained using GC/MS are more precise. The lower sensitivity of HN-3 detection using TLC in comparison with GC/MS resulted in fewer options to observe the presence of the low concentration of the analytes. The different value of k_1 at the pH of 7.5, as well as the different half-life of the reaction $t_{1/2}$, are given by the different initial concentrations of the analyte in the buffer, which, as mentioned above, caused at 2 mg mL^{-1} the significant decrease of the pH of the environment and slowing down of the hydrolysis. The half-lifes of the reactions when using both methods correspond.

Out of the stated rate constants, the significant influence of the rate of HN-3 degradation via pH is evident. Franke [8] states that the increasing pH does not have an apparent influence on the hydrolysis of sulphur mustard (bis(2-chloroethyl)sulphide) and that in alkaline environment, it is hydrolysed only by 30% faster. It was believed that with nitrogen mustard, HN-3 results in similar conclusions on the basis of isosterism of nitrogen and sulphur as well as the formation of cyclic intermediate, which allows the subsequent nucleophilic substitution. If the rate constant determined at the HN-3 hydrolysis in the buffer with the pH of 7.5 is designated as the rate constant of neutral environment, then when alkalising the environment to reach the pH of 12.5, the hydrolysis is accelerated by 500%. Alkalising the

Table 3 Rate constants in first step of tris(2-chloroethyl) amine hydrolysis in alkaline environment and half-life of reactions determined by thin-layer chromatography and gas chromatography/mass spectrometry

pH	TLC			GC/MS		
	k_1 (min^{-1})	SD (min^{-1})	$t_{1/2}$ (min)	k_1 (min^{-1})	SD (min^{-1})	$t_{1/2}$ (min)
7.5	0.011	0.008	60.49	0.025	0.018	27.74
8.5	0.054	0.007	12.88	0.050	0.028	13.98
9.5	0.069	0.015	10.06	0.064	0.025	10.87
10.5	0.111	0.022	6.27	0.078	0.025	8.89
11.5	0.151	0.055	4.58	0.094	0.019	7.39
12.5	0.205	0.103	3.37	0.124	0.012	5.60

environment results, besides other things, in increasing its polarity, which determinatively influences the formation of aziridinium cation.

Bartlett and Swain [28] determined the rate constant of 0.155 ± 0.01 min^{-1} of the first order of the first step of bis(2-chloroethyl)sulphide hydrolysis. When comparing with Table 3, it is apparent that HN-3 hydrolysis contrary to the significant acceleration in alkaline environment, it does not reach the rate of bis(2-chloroethyl)sulphide hydrolysis.

4.8 Influence of temperature increase on HN-3 degradation

The rate of HN-3 degradation at the higher temperature was observed. The degradation in the pH environment of 8.5 at the temperature of 25 °C and the decomposition in the same environment at the temperature of 40 °C were compared. HN-3 in the sample at the higher temperature after 15 min was not detected using GC/MS. The rate constant 0.190 ± 0.014 min^{-1} (3.8 × rate increase) was determined. The reaction half-life was 3.65 of a minute. The combination of high temperature and mildly alkaline pH resulted in rapid HN-3 degradation.

By determining the velocity constants at various temperatures, it was possible to determine the frequency factor A and the activation energy E_A in the Arrhenius equation:

$$k = A \cdot e^{\frac{-E_A}{RT}} \quad (3)$$

The activation energy of the first step of hydrolysis E_A is according to the ascertained values equal to 69,086 $J \cdot mol^{-1}$. The frequency factor is then $6.352 \cdot 10^{10}$ min^{-1}. It is possible to utilise the calculated values to deduce the rate constants of the first step of HN-3 hydrolysis at various temperatures.

5 Conclusion

Tris(2-chloroethyl)amine hydrolyses in three steps when forming an electrophilic intermediate which is created in a polar environment. The dependence of hydrolytic degradation of tris(2-chloroethyl)amine on pH was proven. In the neutral environment of the pH of 7.5, the first step of hydrolysis, the transformation to *N*,*N*-bis(2-chloroethyl)-*N*-2-hydroxyethylamine, happened in the period of 300 min and in the concentrated sample in 1500 min. The HN-3 concentration plays an important role mainly in the neutral values of pH, which, due to the influence of acid formation, decreases under the pK_a the values of hydrolysed basic compounds. The increasing value resulted in the significant acceleration of HN-3 hydrolysis. The rate constant of the first step of hydrolysis combined with the increase of pH from 7.5 to 12.5 increased on the 500% of the original value.

It was proven that to quantitatively determine the hydrolysis kinetics, GC/MS as well as TLC can be used; however, TLC is characterised by higher result deviations and lower sensitivity. By selecting a suitable derivatisation reagent, all determined analytes can be observed at the same time. It can be utilised as a suitable technology to detect and determine the hydrolytic products of tris(2-chloroethyl)amine degradation. To determine the substances using GC/MS, the evaporation of water layer, dissolving the residue in a non-water solution, and the derivation before spraying into a distillation column are required. The hydrolysis itself is influenced during the steps.

The determined values are the contribution for the decontamination practice of chemical warfare agents, because the accessible sources inform about the unsuitability of alkaline hydrolysis for the decontamination of nitrogen mustards, even though the warnings are not supported by kinetic data. The constants to calculate the Arrhenius equation of the dependence of the rate of tris(2-chloroethyl)amine degradation at various temperatures were also determined in pH 8.5.

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Compliance with ethical standards

Conflict of interest The author declares no conflicts of interest.

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