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Kinetic investigation on aqueous decomposition of 2-chloroethylnitrososulfamide

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Abstract—The kinetics decomposition of 2-chloroethylnitrososulfamides (CENS) was studied in aqueous buffered solutions with pH ranging from 0 to 14. The study was monitored by RP-LC–MS and conventional UV spectrophotometry. The reaction proceeded via a pseudo-first-order kinetic with significant correlation coefficient. The major decomposition products from CENS after incubation in phosphate buffer were isolated and identified by NMR and mass spectrometry. The results indicate that the mechanism pathway involves a denitrosation of the CENS and competitive hydrolysis with nucleophilic attack on the sulfur atom and formation of sulfamate compounds.

2-Chloroethylnitrososulfamides (CENS) are promising alkylating agents which have focused our interest over the past decade due to their better biological profile compared to their structural parent, the chloroethylnitrosoureas (CENU).[1–8] Indeed, in contrast to CENU, this class of compounds does not have the ability to generate carbamoylating species which are known to be involved in the toxicity of CENU.[3]

To progress to clinical evaluation, information are needed on the stability and mechanism of decomposition of CENS. So, to gain insight into the stability of this family of compounds, we have carried out the first experimental investigation on the fragmentation of CENS in aqueous media by LC–MS and conventional UV spectrophotometry.

For this study, three CENS of interest were selected (Fig. 1) considering their better activity in vitro on A549 and MCF7 cell lines, compared to CENU.[9–11]

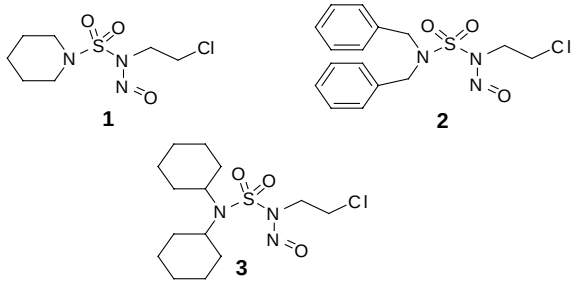

Figure 1. CENS used in this study.

In a first approach, we monitored by TLC the decomposition of the different CENS in buffer solution. The first observation indicated that CENS lost their nitroso group to give two compounds with different R_f.

The kinetics of decomposition for the three different compounds were experimentally determined by UV spectrophotometry, analyzing evolution of the spectra according to the time of 10^{-4}–10^{-5} M solutions in buffer solutions with pH ranging from 0 to 14. Unless otherwise stated, the reactions were monitored at 291 K, the ionic strength being adjusted (0.2 M) with addition of KCl.

Keywords: 2-Chloroethylnitrososulfamide; Alkylating agent; Anticancerous compounds; Kinetic decomposition; LC–MS; UV spectroscopy.
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For the decomposition of CENS, three distinct types of reactivity can be distinguished. First in basic solution, the decomposition of the compounds **1**, **2** and **3** takes place in only one step with hypsochromic shift and appearance of an isobestic point (Fig. 2).

For all the compounds studied at acidic pH interval, the decomposition of the CENS occurred via independent step with a decreasing of absorbance in time. This observation can be attributed to the loss of chromophoric nitroso group.

The way of decomposition of the three compounds in neutral pH interval was quite different. First, we observed a slow decrease in absorbance with hypsochromic shift and apparition of new absorption band which increase according to the time. The reaction in neutral pH is more complex. As we can see in Figure 4, the decomposition occurred probably via two competitive steps. The slow decrease in absorbance value can be attributed to the denitrosation phenomena which correspond to the reaction in acidic media (Fig. 3). The increase in absorbance and hypsochromic shift accompanied with apparition of isobestic point near 254 nm was similar to the decomposition in basic medium (Fig. 2).

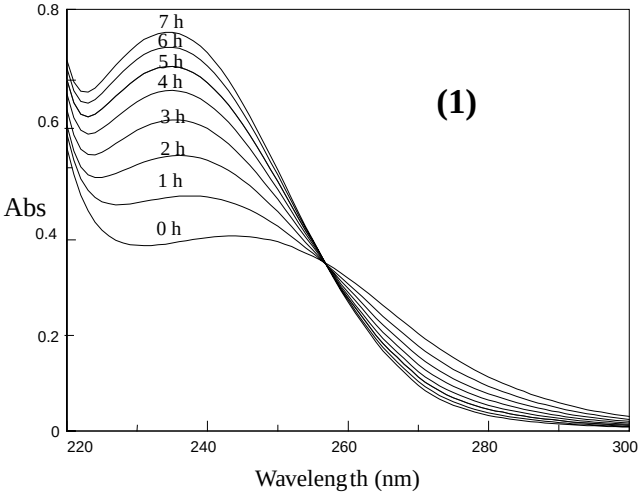


Figure 2. Evolution of 10^{-4} M solution of compound **1** at pH 8.6.

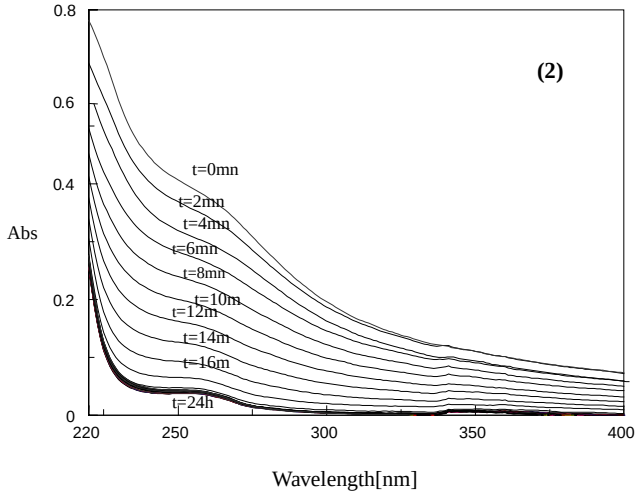


Figure 3. Evolution of 10^{-4} M solution of compound **2** at pH 0.14.

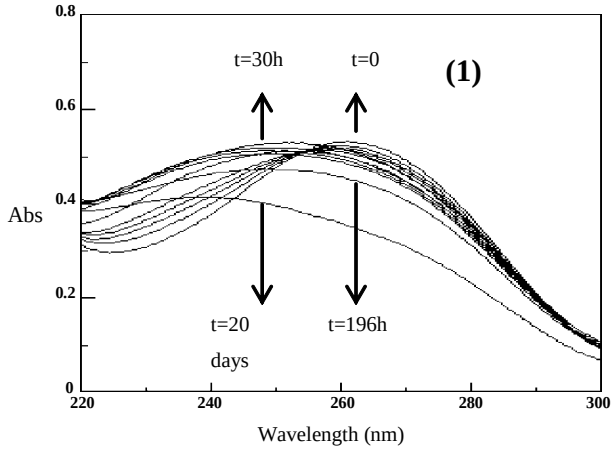


Figure 4. Evolution of 10^{-4} M solution of compound **1** at pH 7.4.

For the decompositions taking place in only one step, the values of the observed rate coefficients (K_{obsd}) have been determined graphically using the function $\ln[(A - A_\infty)/(A_0 - A_\infty)] = f(t)$ for the acidic medium and $\ln A = f(t)$ for the basic medium. Linearization of the experimental data showed that these decompositions are excellent pseudo-first-order reactions. The coefficients of linear regression are superior to 0.98 for all compounds. Absolute values of the slopes represent the rate constant of the reaction of the pseudo-first-order. For neutral pH, the decomposition reactions of the compounds **1**, **2** and **3** are competitive and the treatment of kinetic data revealed pseudo-first-order. To increase the precision of calculation of the rate constants, a sufficient number of experimental points situated in the first time of half-reaction of each process have been used. For the first stage 20 cycles of 90 s have been recorded and 25 cycles of 900 s for the second. For all pH values, the rate constant (K_{obsd}) and the times of half-life have been determined.

The relation between the logarithms of the observed that pseudo-first order rate constants and the pH are represented in the following Figure 5.

The U-shaped profile is a characteristic of the reaction catalyzed in acidic or basic medium as already noted for analogues compounds.[12–20]

For each compound, three distinct regions of reactivity can be distinguished. For the three compounds, the maximum stability was observed for the pH interval ranging from 5 to 8. As shown in Figure 5, the presence of H_3O^+ or OH^- catalyzes the hydrolysis of these compounds.

For the studied compounds and for a given pH, R^1 and R^2 have a moderate influence on the values of the decomposition constants.

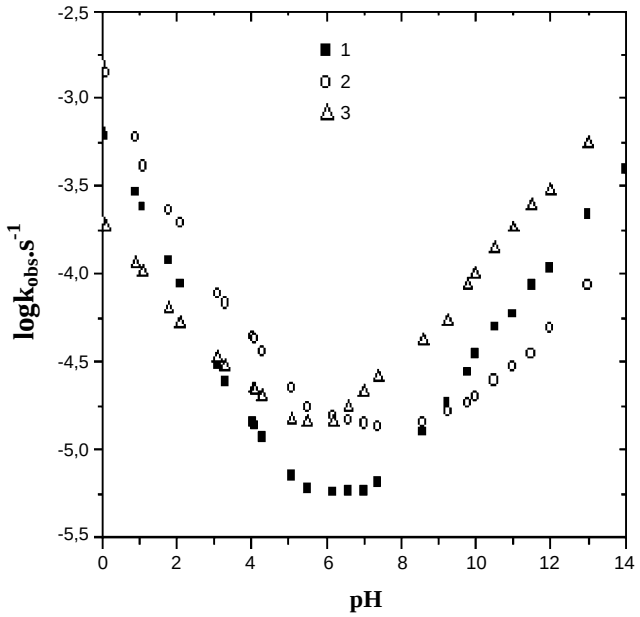


Figure 5. Plot of log K_{obsd} versus pH.

The kinetic of decomposition of compounds **1**, **2** and **3** was monitored at pH 7.4; $T = 37$ °C by RP-LC–MS.[34] The evolution of the chromatograms is depicted in Figure 6. We observed a disappearance of CENS with formation of two main products (Table 1).

The first single signal corresponds to the starting product. After decomposition, there are two components eluting earlier at 2 and 3 min. The main decomposition products were tentatively identified by LC–MS; data are shown in Table 2.

Initially, compounds **1**, **2** and **3** have been identified by HPLC–MS. They have shown, respectively, a retention time at 9.26, 12.44 and 5.5 min detected with UV-PDA detector at $\lambda = 253.46$, 251.44 and 244.64 nm. Their mass spectrum revealed an ion molecular peak at 278.12 $[M+Na]^+$ for compound **1**, 390.01 $[M+Na]^+$ for compound **2** and 352.44 $[M+H]^+$ for compound **3**.

After decomposition, the total ion chromatograms (TIC) contain at least two components with nearly identical retention times for all compounds used in this

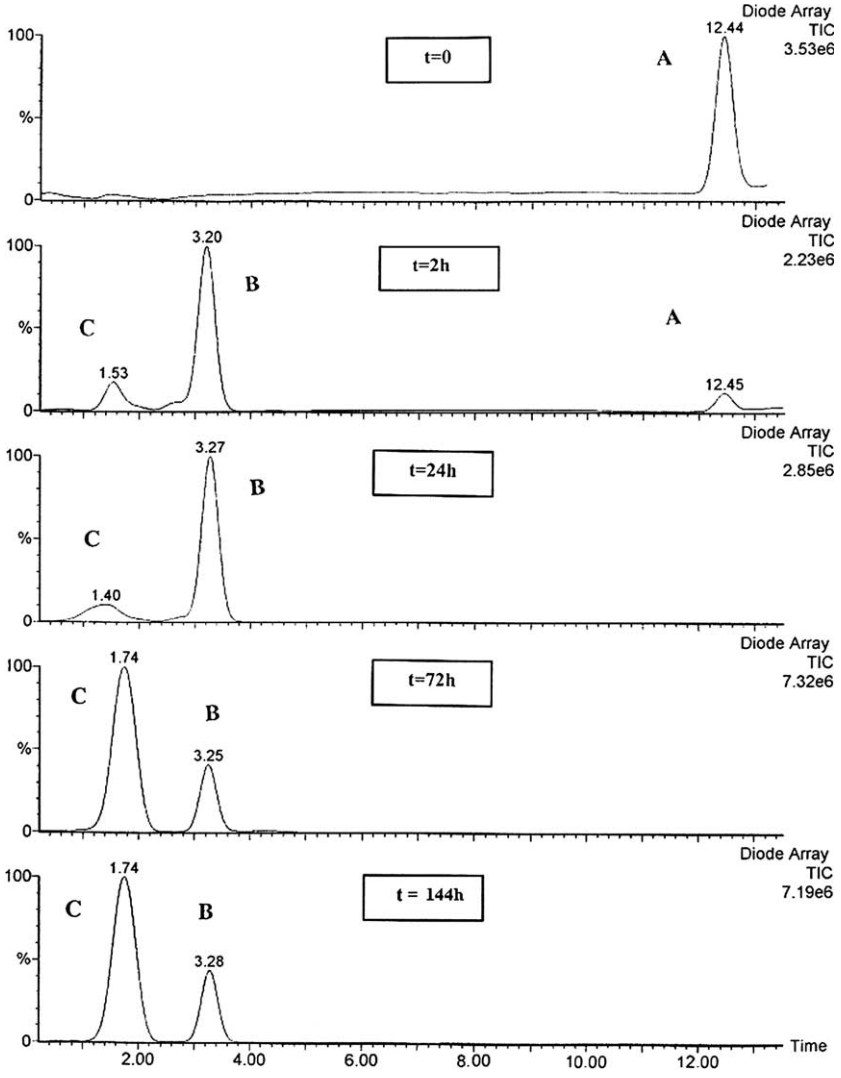


Figure 6. Evolution according to time of HPLC chromatograms of compound **3**.

Table 1. Rate constants and half-life of decomposition of CENS at different pH

	pH	0.1	2.1	4.1	6.2	7.4	8.6	9.25	10.0	11.5	13.0
1	$K_{(obsd)} \times 10^4$, s^{-1}	6.114	0.876	0.137	0.057	0.065	0.125	0.183	0.350	0.858	2.185
	$t_{(1/2)}$, min	19	132	868	2003	1777	917	632	329	134	53
2	$K_{(obsd)} \times 10^4$, s^{-1}	14.00	1.960	0.420	0.150	0.137	0.142	0.163	0.200	0.355	0.865
	$t_{(1/2)}$, min	8	59	273	750	841	817	705	576	325	133
3	$K_{(obsd)} \times 10^3$, s^{-1}	1.857	0.521	0.218	0.145	0.260	0.419	0.537	1.002	2.433	5.480
	$t_{(1/2)}$, min	62	221	530	786	444	275	215	115	48	21

Table 2. Mass spectral and HPLC chromatographic characterization of the products resulting from aqueous decomposition of CENS after 72 h in phosphate buffer at 37 °C

Compound	UV detection		ESI mass detection *m/z* (positive mode)	
	T_r (min)	UV data (nm)	T_r (min)	Masse
1	3.33	253.26	3.32	279.15
	2.58	236.47	2.59	227.15
2	9.33	251.46	9.26	390.01
	3.14	265.46; 226.46	3.02	279.06; 301.02 101.79
	2.17	268.26	2.09	339.12
3	3.25	265.44	3.15	279.15; 301.09
	1,74	256.46	2.07	345.29

study. LC-ESI-MS data in Table 2 show that both compounds eluting near 2 min have been detected, respectively, by an on-line UV-PDA detector at $\lambda = 236.4$, 268.26 and 256.44 nm for the decomposition of compounds **1**, **2** and **3**. Their mass spectrum showed, respectively, a molecular ion peak at *m/z* 227 $[M+H]^+$, *m/z* = 339.12 $(M+H)^+$ and *m/z* = 345.29 $[M+Na]^+$. They were identified as denitrosated CENS, for example, the chloroethylsulfamide.

The situation of the second product B is somewhat more complex; ESI-MS detection data showed the same values for the three compounds with *m/z* = 279 $[M+H]^+$ and *m/z* = 301 $[M+Na]^+$. The problem was to identify the common species which is eluted near 3 min. It should be noted that this product of decomposition was stable. After isolation, it was identified on the basis of the NMR and mass spectrum.

The kinetic parameters of the decomposition of the CENS under aqueous condition were determined by following the disappearance of HPLC–UV peak of the starting compound and plotting the peak area of each CENS versus time (Fig. 7). The experimental data were fitted using an exponential model (data not shown). The half-life values were calculated by a mathematical model illustrating pseudo-first-order kinetics: $C = C_0 \exp(-Kt)$, where C_0 represents the initial concentration of the CENS, C, the concentration at time t, and t time of incubation (hours). The observed rate constants and half-lives, calculated by $\ln 2/K$, in aqueous solution are given in Table 3.

The rate constants and half-life determined and compared to some CENU[14–33] showed that CENS are more stable; however, compound **3** is somewhat not stable and its half-life exhibited a comparable value with the CENU.

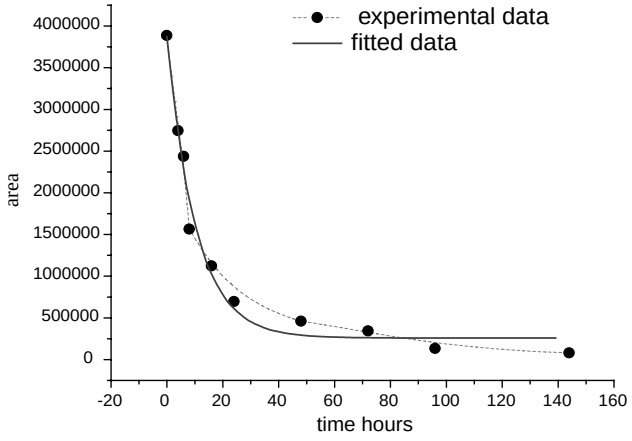


Figure 7. Plot of peak area versus time of decomposition of compound **2**.

Table 3. Decomposition kinetic parameters of CENS at pH 7.4; $T = 37$ °C

Compound	K_{obsd} min^{-1}	r^2	$t_{1/2}$[a] min
1	0.0060	0.997	115.0
2	0.0016	0.983	424.5
3	0.0339	0.989	20.40
BCENU	0.0133	—	52.00[33]
CCNU	0.0144	—	48.00[14]

[a] The variability of triplicate samples was no more than 4%.

HPLC analysis of the residue obtained after complete decomposition of CENS showed the formation of two degradation products (Fig. 7). These products have been tentatively identified using the following data:

Product (C) was identified comparing the chromatographic feature (Fig. 8) of the synthesized product. The NMR and mass spectrometry showed that this compound is the chloroethylsulfamide. Product (B)

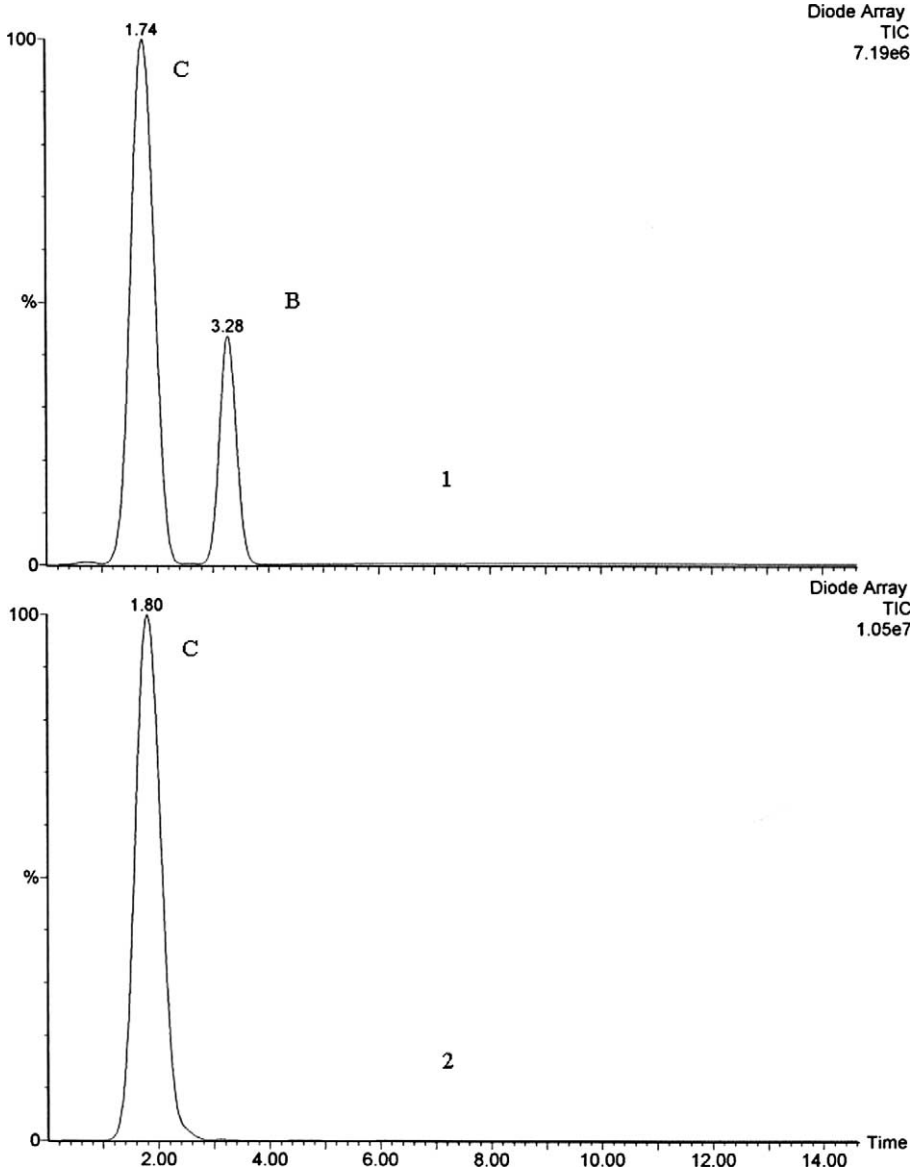
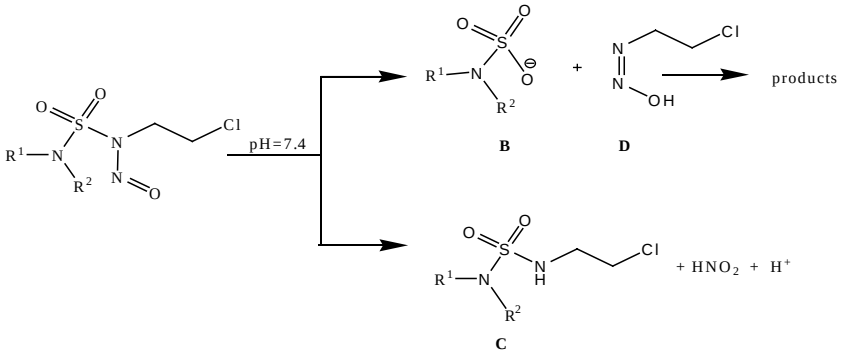
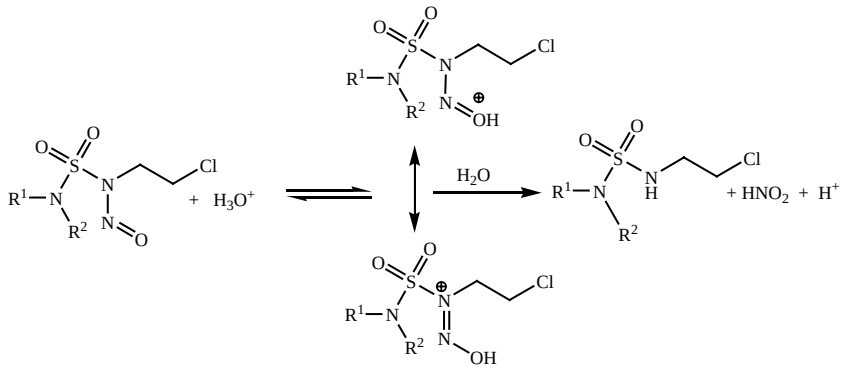


Figure 8. HPLC chromatograms: 1—Evolution of decomposition reaction of compound **3** after 72 h of incubation in phosphate buffer pH 7.4; $T = 37$ °C. 2—Synthetic compound C (*N*-chloroethylsulfamide).



Scheme 1. Proposed mechanism of decomposition.



Scheme 2. Proposed mechanism of denitrosation.

was identified as sulfamate by ESI-MS and NMR spectrometry.[34]

We can propose the following decomposition mechanism (Scheme 1) in aqueous phosphate buffer. The phenomena implied that the decomposition proceeded via two competitive steps.

The formation of compounds C and B is the result of the denitrosation and competitive hydrolysis of the CENS. The compound D could not be detected because of its high instability in the experimental conditions. Lown and co-workers[12–33] have suggested these species as intermediate in the decomposition of chloroethyl nitrosoureas under physiological conditions. It has been postulated to be the ultimate electrophile responsible for the biological activity of CENU.

The denitrosation mechanism of CENS takes place through the initial protonation of *N*-chloro ethylnitrososulfamide and subsequent nucleophilic attack on the protonated oxygen (Scheme 2). The alkaline hydrolysis step occurs via a nucleophilic attack of OH^- on the sulfur atom to form sulfamate species B and chloroethyldiazohydroxide D. The same process has been noted for the same type of nitroso compounds such as MNTS (*N*-methyl-*N*-nitroso-*p*-toluene sulfonamide) and MNNG (*N*-methyl-*N*-nitro-*N*-nitrosoguanidine).[32]

On the other hand, the spectrophotometric study indicates that in acidic media the decomposition occurs via independent step and corresponds to the denitrosation reaction. In basic pH, compounds undergo hydrolysis but not denitrosation.

In conclusion, this study constitutes the first decomposition study of three CENS in aqueous buffer solutions. It revealed that these compounds decompose via a general acido-basic catalysis as previously noted for nitrosoureas. The LC–MS study at physiological pH and temperature showed a formation of at least two products which have been identified.

Acknowledgments

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34. Experimental information: *UV spectrophotometry*: The kinetics were monitored spectrophotometrically with a UV–vis double beam spectrophotometer Jasco (Japan) model (V-530) connected to a PC computer with spectral analysis program and equipped with cell compartment thermostated by a Jasco EHCT temperature controller. Sealed quartz cells were used for each experiment. *HPLC system*: The HPLC system consisted of Waters model 2695 separation module with autosampler and a PDA Waters 996 photodiode array detector (starting from 220 to 400 nm). The chromatographic apparatus is controlled by Masslynx version 3.5 software package. The separation of products was achieved on a RP-C18 column (150*2 mm id, 5 μm), protected with guard column with the same materials. The column temperature was set at 25 °C. The mobile phase consisted of two solvents: A (water 0.1% formic acid) and solvent B (acetonitrile 0.1% formic acid). The flow rate was 0.25 ml/min. A linear gradient elution was performed as follows: 0 min, 40% A, 60% B; 10–20 min, 100% B; 20.10 min, 40% A, 60%; 20.10 to 30 min, 40% A, 60% B. The volume of injection into the column was 50 μl, and the injector syringe was washed with 50/50 methanol–water grade HPLC before each injection. The compounds were detected using Waters (Micromass ZQ) single quadrupole mass spectrometer equipped with electrospray ionisation (ESI) source. The MS was operated in the positive ion mode. *General procedure for analysis of products of aqueous decomposition of chloroethylnitrososulfamides:* A solution 2.10^{-5} mol dm^{-3} of the chloroethylnitrososulfamides in 100 mM sodium phosphate buffer (pH 7.4) was incubated at 37 °C in vial equipped with Teflon septum. Each compound was dissolved in the minimum of acetonitrile and diluted with the phosphate buffer. The mixture was vortexed for 10 s and the aliquots were removed at intervals and injected for analysis into RP-HPLC-MS system. *Isolation of the products resulting from the decomposition of the CENS:* A mixture of CENS (200 mg) and pH 7.4 phosphate buffer 2% acetonitrile 50 ml was stirred at 37 °C in reaction vials equipped with Teflon septum. The reaction was monitored by TLC and showed the appearance of two new spots of different R_f. The reaction was stirred until total disappearance of the starting material and then diluted with dichloromethane. The mixture was washed with saturated aqueous solution of NaCl. The organic phase was separated and dried on anhydrous sodium sulfate and concentrated under reduced pressure to lead to a yellow oil. The products of the decomposition were then separated on a silica gel column eluted with dichloromethane. Compound **1** (B) ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.55 (m, 6H, CH_2 cycl); 2,74 (q, 2H, N-CH_2); MS ESI$^+$ 25 eV m/z = 187.29 $[M+Na]^+$ (100%). Compound **1** (C): ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.62 (t, 1H, NH); 3.64 (t, 2H, Cl-CH_2); 3.40 (q, 2H, N-CH_2); 3.32 (t,4H, N-CH_2 cycl); 1.62–1.50 (m, 6H, CH_2 cycl); MS ESI$^+$ 20 eV: m/z = 227.24 $[M+H]^+$ (85%). Compound **2** (B) ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.86 (s, 4H, $2CH_2$Bn); 7.26 (m, 10H, 2ArH); MS ESI$^+$ 25 eV: m/z = 300.08 $[M+Na]^+$ (65%). Compound **2** (C): ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.45 (m, 10H, 2ArH), 4.35 (t, 1H, NH); 4.35 (s, 4H, $2CH_2$Bn); 3.60 (t, 2H, $ClCH_2$); 3.25 (q, 2H, N-CH_2); MS ESI$^-$ 20 eV: m/z = 337.99 $[M-H]^-$ (100%). Compound **3** (B) ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.57 (q, 2H, N-CH_2); 1.44 (m, 20H, CH_2 cycl); MS ESI$^+$ 40 eV: m/z = 283.18 $[M+Na]^+$ (100%). Compound **3** (C) ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.45 (t, 1H, NH); 3.70 (t, 2H, $ClNH_2$); 3.35 (q, 2H, N-CH_2); 3.25 (m, 2H, 2N-CH); 1.75–1.00 (m, 20H, CH_2 cycl); MS ESI$^+$ 40 eV: m/z = 345.29 $[M+Na]^+$ (100%).