Study of the Degraded Products of *N*-Chloroserine in Chlorinated Waters Using High-Performance Liquid Chromatography

J.J. Lamelas and †**J. Franco**
Departamento de Química Física y Orgánica, Universidade de Vigo, Facultad de Ciencias, As Lagoas s/n. 32004 Ourense, Spain

J.M. Antelo
Departamento de Química Física, Universidade Santiago de Compostela, Facultad de Químicas, Santiago de Compostela, Spain

M. Varela
Departamento de Química Inorgánica, Universidade de Vigo, Facultad de Ciencias, As Lagoas s/n. 32004 Ourense, Spain

E. Falqué-López*
Departamento de Química Analítica y Alimentaria, Universidade de Vigo, Facultad de Ciencias, As Lagoas s/n. 32004 Ourense, Spain

Abstract

The most widely used method for identification of decomposed products of *N*-chloroamino acids is the qualitative 2,4-dinitrophenylhydrazine method. However, this method has serious drawbacks. For this reason, a new method based on high-perfomance liquid chromatography that permits the identification and quantification of the reaction products is proposed. Included in this study is the optimization of the method (linearity, repeatability, reproducibility, and recovery) and its application to *N*-chloroserine, the use of which can be easily extended to other amino acids (the L-serine being a simple structure amino acid that has proved to be plentiful in both drinking and residual water). To study the degradation products of *N*-chloroserine, an ion-exchange capillary column and detection by refractive index coupled with ultraviolet detection at 210 nm have been used. The compounds determined are glycolaldehyde and β-hydroxypyruvic acid. This identification is carried out in different mediums because the predominance of one product or another depends on the pH value. Finally, it is shown that the determined products are coherent with the reaction mechanism proposed for the process.

Introduction

The presence of amino acids in natural and residual water is caused by the hydrolysis of the proteins that react in the medium. When these waters are treated with hypochlorite for potability, chloro-amino acids are formed. These compounds are unstable, and during degradation, they can originate aldehydes, ketones, nitriles, or ketoacids (depending on the conditions in which decomposition occurs), generating disagreeable odors and flavors in the water (1) and also causing problems in aquaculture (2).

The kinetic processes of these substances have been widely studied by many authors (3–7), but investigations centered on the study of

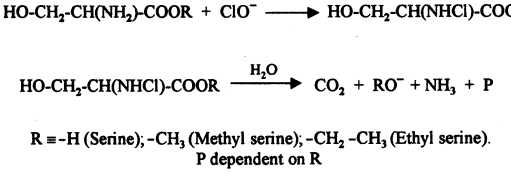

Figure 1. Scheme of the decomposition reaction for *N*-chloroserine and its esters.

Table I. Study of the Repeatability of the Method by HPLC for Glycolaldehyde with Detection by Refractive Index

C^*	$C^{e\dagger}$	RSD‡	%RE§
0.0100	0.0100	1.6	1.4
0.0050	0.0049	1.4	1.4
0.0025	0.0027	1.4	1.2
0.0010	0.0012	4.4	3.7
0.0008	0.0007	7.6	6.4

* *C*, real concentration (in molarity).
† C^e, experimental concentration (in molarity).
‡ RSD, relative standard deviation.
§ %RE, relative error over average value.

* Author to whom correspondence should be addressed: e-mail efalque@uvigo.es.

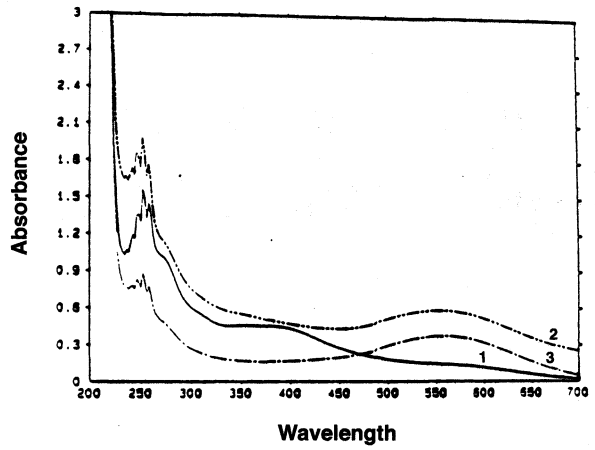

Figure 2. Decomposition products of *N*-chloroserine (1), *N*-chloromethyl-serine (2), and *N*-chloroethyl-serine (3) using the method of 2,4-dinitrophenylhydrazine (study of the extraction with NaOH of the organic phase at 30 min).

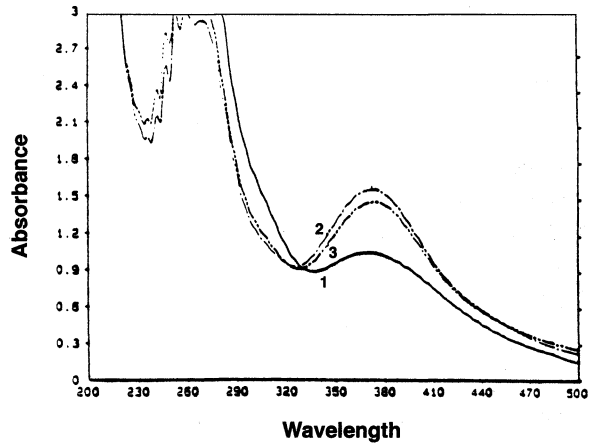

Figure 3. Decomposition products of *N*-chloroserine (1), *N*-chloromethyl-serine (2), and *N*-chloroethyl-serine (3) using the method of 2,4-dinitrophenylhydrazine (study of the aqueous phase at 30 min).

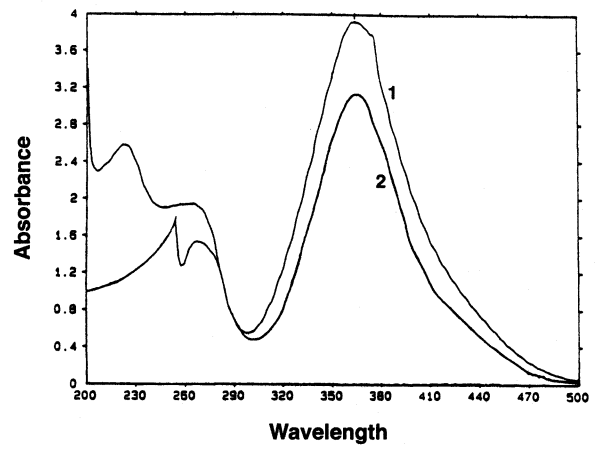

Figure 4. UV–vis spectras for 2,4-dinitrophenylhydrazone of glycolaldehyde (1) and β-hydroxypyruvic acid (2) at 30 min.

the reaction products are scarce and have always been carried out in a qualitative manner.

The amino derivatives react rapidly with the chlorinated agent, leading to the corresponding chlorinated compounds. To be exact, the *N*-chloroserine decomposes to produce β-hydroxypyruvic acid and glycolaldehyde. A diagram of this process is shown in Figure 1.

The traditional method employed for the identification of these products is the 2,4-dinitrophenylhydrazine method (8), which is based on the reaction of this reactant with the organic compounds containing carbonyl groups. The product resulting from the reaction is the 2,4-dinitrophenylhydrazone of the corresponding derivative. By using the described method, the qualitative presence of the previously indicated products has been detected. However, its applicability is limited and is not without drawbacks, some of which are described here.

First, an extraction must be carried out using benzene with a pH higher than 11, with the possibile consequence of an alteration of the final products of the reaction.

Second, the small modifications of the lateral chain of the corresponding 2,4-dinitrophenylhydrazone produce very small alterations in the maximum absorption value. One can affirm that the wavelength at which the absorption maxima appear does not fundamentally depend on the length of the side chain which, regretfully,

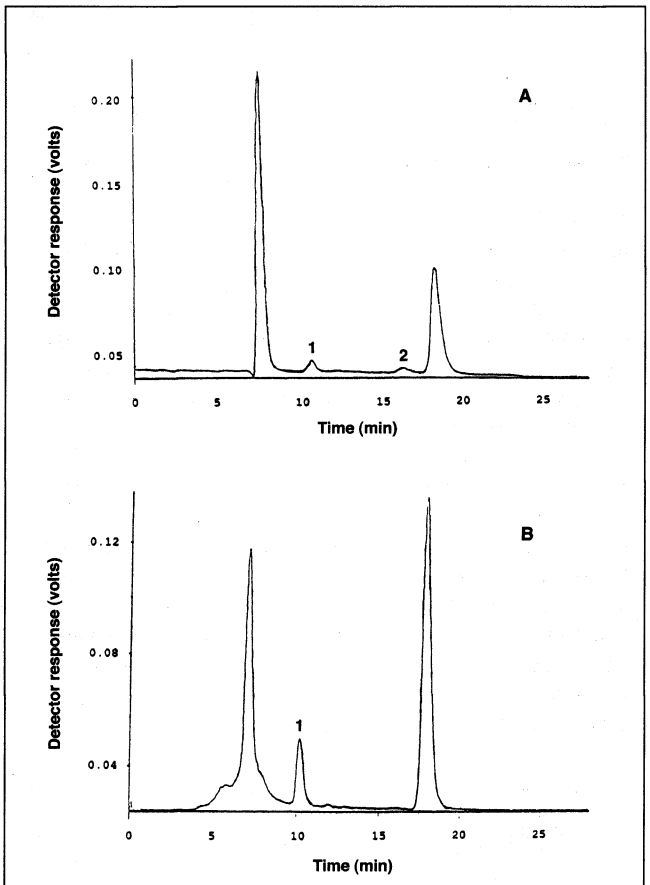

Figure 5. Chromatograms of the decomposition products of *N*-chloroserine in accordance with the procedure as described by refractive index (A) and UV at 210 nm (B). Conditions: mobile phase, 10^{-3}M sulfuric acid; flow rate, 0.5 mL/min; column temperature, 75°C; acetic–acetate buffer, pH 6.03. Peaks: 1, β-hydroxypyruvic acid; 2, glycolaldehyde.

is what we are most interested in. These factors, problematic in themselves, are aggravated in the case of *N*-chloroserine by the existence of an isomeric *cis*-transition in the β-hydroxypyruvic acid and thus the consequent modification of the wavelength of the corresponding 2,4-dinitrophenylhydrazones.

Third, the existence of a variation in the absorption intensities of the 2 maximums found in 2,4-dinitrophenylhydrazones, depending on the pH, has been verified.

Fourth, the results obtained for both the aqueous and organic phase are qualitative.

Fifth, in the case of the serine esters, the results are deceptive. Even though β-hydroxypyruvic acid is detected in the aqueous phase, it is because of a process of hydrolysis of the methyl or ethyl ester of the indicated acid according to whether one refers to the methyl-serine or to the ethyl-serine.

Methods for derivatization of organic *N*-chloroamines and their analysis by high-perfomance liquid chromatography (HPLC) has been described (9–11). The aim of the present work was the optimization of the chromatographic method by HPLC without previous derivatization, which permits the qualitative and quantitative analysis of the decomposition products and their proportion in different mediums.

Experimental

2,4-dinitrophenylhydrazine method

The qualitative method proposed by Cooper et al. (8) consists of obtaining the corresponding 2,4-dinitrophenylhydrazones. For this purpose, the buffer KH_2PO_4–K_2HPO_4, NaCl, and ClO^- were added to the *N*-chloroamino acid or to its esters. When the reaction time had passed, 2,4-dinitrophenylhydrazine was added, and after 30 min, it was extracted using benzene. The aqueous and organic phases, extracted using NaOH, were studied by spectrophotometry using a Beckman (Fullerton, CA) DU-70 apparatus.

HPLC method

The chlorinated water sample was analyzed by HPLC using a Waters chromatographic system (Millipore, Milford, MA) equipped with an automatic injector (model 712), a pump (model 510), and an Aminex (Bio-Rad Labs, Richmond, CA) HPX-87H ion-exchange column (300 × 7.8 mm) placed in a thermostatic bath at 75°C. Sulfuric acid (10^{-3}M) was used as the mobile phase at a flow rate of 0.5 mL/min. The coupling in series of a model 481 ultraviolet (UV) detector (at 210 nm and 0.2 AUFS) and a model 410 refractive index detector (the control and sample cells were maintained at 45°C) was

Table II. Study of the Repeatability of the Method for HPLC for β-Hydroxypyruvic Acid with Detection by Refractive Index and UV (210 nm)

	$C^{e\dagger}$		RSD‡		%RE§	
C*	RI	UV	RI	UV	RI	UV
0.0100	0.0101	0.0099	1.0	1.0	0.8	0.8
0.0050	0.0048	0.0052	0.8	2.0	0.7	1.7
0.0025	0.0025	0.0025	1.5	1.5	1.3	1.3
0.0010	0.0009	0.0009	3.8	5.9	3.2	5.0
0.0005	0.0005	0.0005	7.6	7.6	6.3	6.3

* C, real concentration (mol/L).
† C^e, experimental concentration (mol/L).
‡ RSD, relative standard deviation.
§ %RE, relative error over average value.

Table III. Study of the Reproducibility of the Method for HPLC for Glycolaldehyde and β-Hydroxypyruvic Acid

	Detector	C (mol/L)	RSD*	%RE†
Glycolaldehyde	RI	10^{-2}	0.8	0.8
		10^{-3}	1.0	7.1
β-Hydroxypyruvic acid	RI	10^{-2}	1.5	1.2
		10^{-3}	4.7	3.4
	UV	10^{-2}	1.0	0.9
		10^{-3}	1.3	1.5

* RSD, relative standard deviation.
† %RE, relative error over average value.

Table IV. Study of the Accuracy of the Method for HPLC*

	Detector	*N*-Chloro-serine (mol/L)	*N*-Chloromethyl-serine (mol/L)	*N*-Chloroethyl-serine (mol/L)
Glycolaldehyde	RI	$7.19 \cdot 10^{-3}$	$5.17 \cdot 10^{-3}$	$5.11 \cdot 10^{-3}$
β-Hydroxypyruvic acid	RI	$6.82 \cdot 10^{-3}$	$4.86 \cdot 10^{-3}$	$5.25 \cdot 10^{-3}$
	UV	$6.86 \cdot 10^{-3}$	$4.80 \cdot 10^{-3}$	$5.17 \cdot 10^{-3}$

* The two products were added in $5.00 \cdot 10^{-3}$M.

Table V. Average Concentrations (mol/L) of Glycolaldehyde formed in the Decomposition of *N*-Chloroserine as a function of the pH

Acetate buffer		Borate buffer	
pH 4.09	pH 6.03	pH 7.35	pH 8.81
$3.02 \cdot 10^{-3}$	$2.47 \cdot 10^{-3}$	$2.16 \cdot 10^{-3}$	$1.71 \cdot 10^{-3}$
$3.05 \cdot 10^{-3}$	$2.55 \cdot 10^{-3}$	$2.15 \cdot 10^{-3}$	$1.63 \cdot 10^{-3}$
$3.04 \cdot 10^{-3}$	$2.52 \cdot 10^{-3}$	$2.19 \cdot 10^{-3}$	$1.66 \cdot 10^{-3}$
$3.04 \cdot 10^{-3}$	$2.51 \cdot 10^{-3}$	$2.17 \cdot 10^{-3}$	$1.64 \cdot 10^{-3}$
$3.03 \cdot 10^{-3}$	$2.47 \cdot 10^{-3}$	$2.15 \cdot 10^{-3}$	$1.69 \cdot 10^{-3}$
$3.04 \cdot 10^{-3} \pm 0.01$	$2.50 \cdot 10^{-3} \pm 0.03$	$2.16 \cdot 10^{-3} \pm 0.01$	$1.67 \cdot 10^{-3} \pm 0.02$

used for the detection of the compounds. A model 820 workstation was employed for processing the signal using calibration by an external standard solution.

In all cases and for both methods, the reactives were of the highest level of purity available (Merck, Darmstadt, Germany and Aldrich, Milwaukee, WI), and the preparation of the mobile phase and the standard solutions were carried out in double-distilled water.

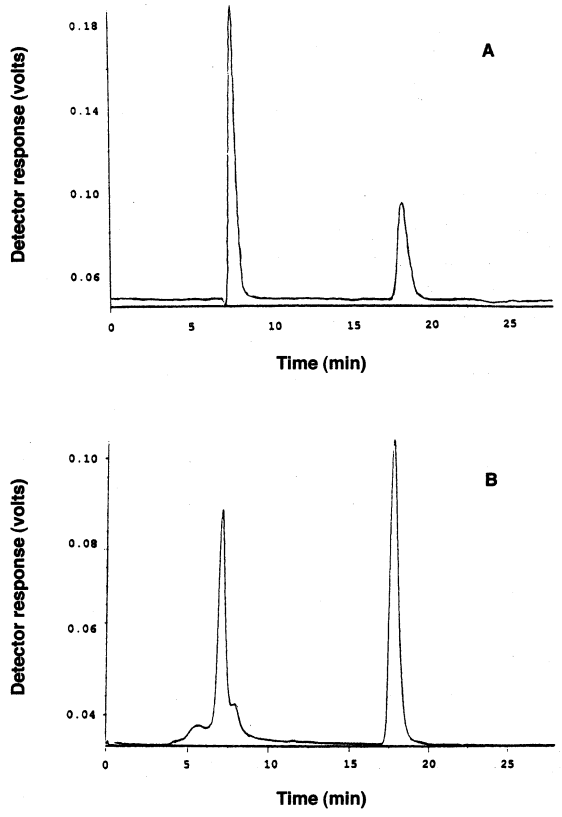

Figure 6. Chromatograms of the decomposition products of *N*-chloromethyl-serine in accordance with the procedure as described by refractive index (A) and UV at 210 nm (B). Conditions: mobile phase, 10^{-3}M sulfuric acid; flow rate, 0.5 mL/min; column temperature, 75°C; acetic–acetate buffer, pH 6.03.

Results and Discussion

Study using UV–visible detection

Using the traditional method of 2,4-dinitrophenylhydrazine (8), glycolaldehyde and β-hydroxypyruvic acid were identified as decomposition products of *N*-chloroserine (Figures 2 and 3).

In Figure 2, the spectrum of the organic phase extracted using NaOH is shown. It can be seen that there is a maximum of absorption at 370 nm for 2,4-dinitrophenylhydrazone of the *N*-chloroserine and at 558 nm for the corresponding derivative of the *N*-chloroesters. In the study of the aqueous phase (Figure 3), the 2,4-dinitrophenylhydrazone of the *N*-chloroserine shows a maximum at 370 nm, and that of the *N*-chloroesters shows a maximum at 374 nm. After corroborating that both the 2,4-dinitrophenylhydrazone of the glycolaldehyde and the β-hydroxypyruvic acid show absorption maxima at 370 nm (Figure 4), it was concluded that the appearance of these compounds was as decomposition products of the *N*-chloroserine but not of the *N*-chloroesters.

Study using HPLC

Optimization of the chromatographic conditions

Following the previous studies, it was concluded that the optimum conditions of separation, identification, and quantitation of the previously mentioned degradation products were as follows: mobile phase, filtered (0.45 µm) and degassed 10^{-3}M sulfuric acid; flow rate, 0.5 mL/min; ideal column temperature, 75°C.

Although it would be sufficient to use the refractive index detector alone, both it and UV have been used serially; the β-hydroxypyruvic acid shows a maximum absorption at 210 nm, permitting the corroboration of the results obtained for this compound (Figure 5).

Linearity

Using the established conditions and injecting 10 µL of standard solutions, a wide linear result was observed that was used to calibrate the system so that the values of the unknown sample concentration (using the integration of the respective peaks as a basis) could be obtained. The calibration was perfomed from 5 different concentrations between 10^{-2} and 10^{-5}M of standard substance; the correlation coefficients were approximately 0.9998 for the refractive index detection of each species and 0.9997 for the UV detection of β-hydroxypyruvic acid at 210 nm.

Table VI. Average Concentrations (mol/L) of β-Hydroxypyruvic Acid Formed in the Decomposition of *N*-Chloroserine as a Function of the pH

RI				UV (210 nm)			
Acetate buffer		Borate buffer		Acetate buffer		Borate buffer	
pH 4.09	pH 6.03	pH 7.35	pH 8.81	pH 4.09	pH 6.03	pH 7.35	pH 8.81
$1.10 \cdot 10^{-3}$	$1.49 \cdot 10^{-3}$	$1.87 \cdot 10^{-3}$	$2.34 \cdot 10^{-3}$	$1.09 \cdot 10^{-3}$	$1.56 \cdot 10^{-3}$	$1.89 \cdot 10^{-3}$	$2.54 \cdot 10^{-3}$
$1.11 \cdot 10^{-3}$	$1.35 \cdot 10^{-3}$	$1.81 \cdot 10^{-3}$	$2.31 \cdot 10^{-3}$	$1.14 \cdot 10^{-3}$	$1.50 \cdot 10^{-3}$	$1.81 \cdot 10^{-3}$	$2.51 \cdot 10^{-3}$
$1.10 \cdot 10^{-3}$	$1.35 \cdot 10^{-3}$	$1.75 \cdot 10^{-3}$	$2.34 \cdot 10^{-3}$	$1.11 \cdot 10^{-3}$	$1.46 \cdot 10^{-3}$	$1.80 \cdot 10^{-3}$	$2.52 \cdot 10^{-3}$
$1.08 \cdot 10^{-3}$	$1.33 \cdot 10^{-3}$	$1.68 \cdot 10^{-3}$	$2.30 \cdot 10^{-3}$	$1.09 \cdot 10^{-3}$	$1.42 \cdot 10^{-3}$	$1.80 \cdot 10^{-3}$	$2.52 \cdot 10^{-3}$
$1.08 \cdot 10^{-3}$	$1.33 \cdot 10^{-3}$	$1.65 \cdot 10^{-3}$	$2.31 \cdot 10^{-3}$	$1.09 \cdot 10^{-3}$	$1.42 \cdot 10^{-3}$	$1.82 \cdot 10^{-3}$	$2.55 \cdot 10^{-3}$
$1.10 \cdot 10^{-3} \pm 0.01$	$1.37 \cdot 10^{-3} \pm 0.06$	$1.75 \cdot 10^{-3} \pm 0.08$	$2.32 \cdot 10^{-3} \pm 0.02$	$1.10 \cdot 10^{-3} \pm 0.02$	$1.47 \cdot 10^{-3} \pm 0.01$	$1.82 \cdot 10^{-3} \pm 0.02$	$2.53 \cdot 10^{-3} \pm 0.02$

Precision

For the study of the repeatability of the method, statistical calculation was applied to the experimental values obtained for each concentration of the standards used in the construction of the calibration curve. Because the number of repetitions in this study was $n = 8$ (for a probability of 95%), Fisher's t value is 2.365, so the relative error over the average value can be calculated. The real concentration values and those from the calibration curve obtained from the analysis of glycolaldehyde and β-hydroxypyruvic acid are displayed in Tables I and II, respectively; the statistical values of the relative standard deviation and the relative error over the average value for each concentration and standard substance are also displayed. In addition, in order to study the reproducibility of the method, 2 different concentrations for each standard substance were elected for the interval in which linearity was studied; during 10 consecutive days, 3 determinations were carried out with each injection of 10 µL under the previously specified working conditions. By applying statistical calculation to the series of values obtained in this manner ($n = 9$, $t = 2.262$), it can be confirmed that the reproducibility was good. The relative standard deviations and relative errors are shown in Table III.

Recovery efficiency

In order to evaluate the recovery efficiency of the method, a known quantity of each standard substance was added to the samples, and the concentration was recalculated. The average values obtained from 8 measurements for each series are shown in Table IV. Because the additions of the standard substances were performed as they appeared, upon completion, at a concentration of 5.10^{-3}M, it was possible to confirm that in the case of the *N*-chloroserine, an important increase in the concentration of both species was produced, thus confirming that they are the degradation products. The important variations were not seen in the case of the *N*-chloroesters because, as was indicated, they do not appear as decomposition products.

Study of the influence of pH in the formation of the decomposition products

Starting with the calibration data, the quantitative determination of the reaction products was carried out. For this, several series at different pH levels were prepared to study the influence in the formation of the products. The reaction medium was prepared as follows: 5 mL of serine, methyl-serine, or ethyl-serine (0.3M) were added to 5 mL of the corresponding buffer (acetic–acetate or boric–borate) (0.4M) and 5 mL of water. Following this, 2 mL of $4.58.10^{-3}$M sodiumhypochlorite was added. It was left to react for 6 h before placing it into the HPLC. Figures 6 and 7 show the type of chromatograms that were originated by the decomposition products of the *N*-chloromethyl-serine and *N*-chloroethyl-serine, respectively. The determined yield of the identified decomposition products is the average of 5 measurements carried out in each series at intervals of 1 h in order to confirm their stability. Tables V–VII display the concentrations and outputs corresponding to the 2 degradation products of the *N*-chloroserine.

In view of the results obtained, it can be concluded that the *N*-chloroserine decomposes, forming glycolaldehyde and β-hydroxypyruvic acid. Also, the pH influences their proportion; the formation of the glycolaldehyde is favored in an acidic medium, whereas in a basic medium, the reaction evolves towards the formation of β-hydroxypyruvic acid. In Figure 8, this influence is evident.

In the same way, the chromatographic study of 2 derivatives of the *N*-chloroserine (*N*-chloromethyl-serine and *N*-chloroethyl-serine) has been carried out employing the same procedures. It can be concluded that neither glycolaldehyde nor β-hydroxypyruvic acid are formed in their decomposition. In experiments carried out in

Table VII. Yield of Products from the Decomposition Reaction of *N*-Chloroserine*

	Acetate buffer		Borate buffer	
	pH 4.09	pH 6.03	pH 7.35	pH 8.81
RGA†	66.4	54.6	47.2	36.5
RHP‡	24.0	32.1	39.1	53.1
RT§	90.4	86.7	86.3	89.6

* Yield was determined as the percentage of reaction product obtained with respect to the stoichiometrich amount calculated on the basis of the limiting reactant.
† RGA, yield in glycolaldehyde.
‡ RHP, yield in β-hydroxypyruvic acid.
§ RT, total yield.

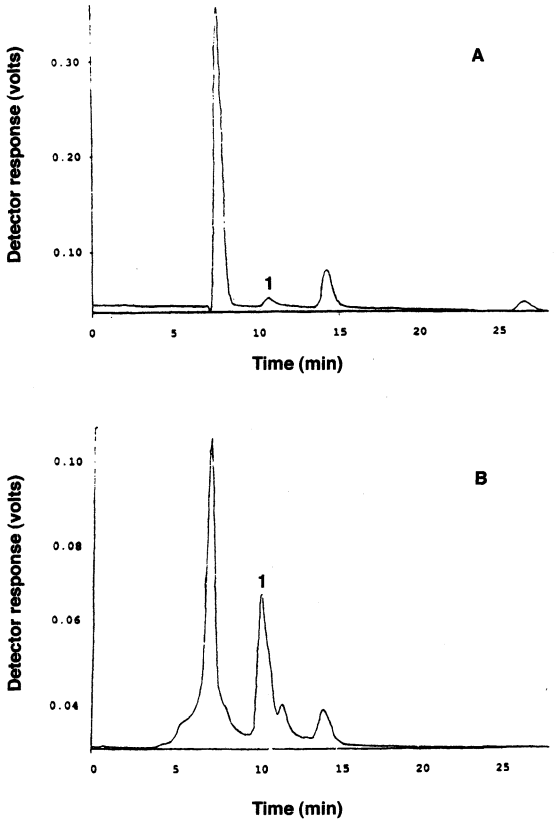

Figure 7. Chromatograms of the decomposition products of *N*-chloroethyl-serine in accordance with the procedure as described by refractive index (A) and UV at 210 nm (B). Conditions: mobile phase, 10^{-3}M sulfuric acid; flow rate, 0.5 mL/min; column temperature, 75°C; boric–borate buffer, pH 9.50. Peaks: 1, β-hydroxypyruvic.

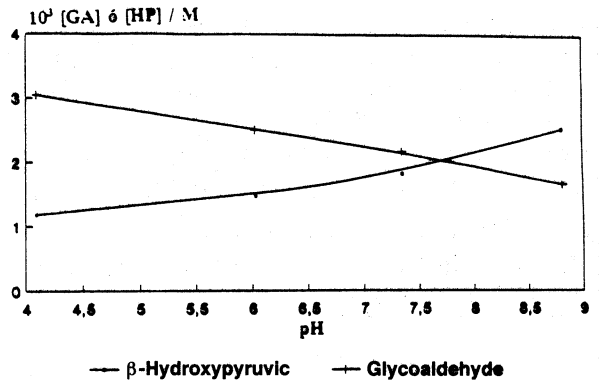

Figure 8. Influence of the pH on the formation of glycolaldehyde and β-hydroxypyruvic acid as degradation products of *N*-chloroserine.

boric–borate buffer at pH 9.5, the appearance of bands at the retention times corresponding to β-hydroxypyruvic acid is observed (Figure 7). This phenomenon is explained by the hydrolysis suffered by the *N*-chloroesters in basic medium; therefore, the presence of this acid is a consequence of the decomposition reaction of the *N*-chloroserine resulting from this hydrolysis. The fact that the bands corresponding to the glycolaldehyde do not appear as well is justifiable if one takes into account that its formation is not favored in basic medium. It is also interesting to note that in some neutral samples that had been alkalized after the decomposition reaction had finished, the presence of β-hydroxypyruvic acid was also detected, which could be explained by the possible hydrolysis of the decomposition products of the *N*-chloroesters that evolve towards the formation of that compound.

Having (as a precedent) the results obtained by HPLC for the *N*-chloroserine, hypotheses were made about the possible degrada-

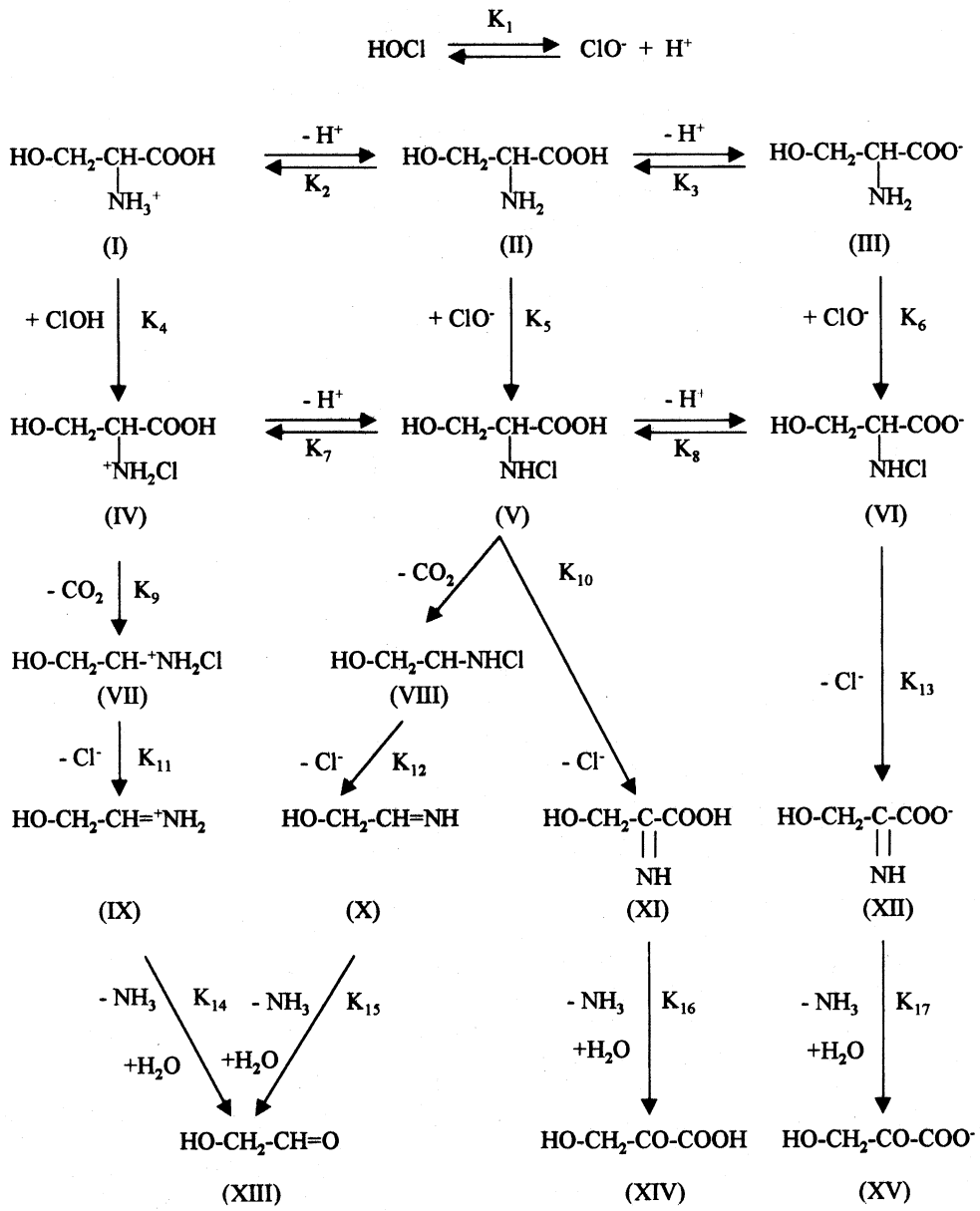

Figure 9. Decomposition mechanism for *N*-chloroserine.

tion products of their *N*-chloroesters. One of these involves the possibility of the formation of hydroxyacetone in a neutral–acid medium and the methyl ester of β-hydroxypyruvic acid in a neutral–basic medium (for the *N*-chloromethyl-serine) and 1-hydroxy-2-buta-none in a neutral–acid medium and the ethyl ester of the β-hydroxypyruvic acid in a neutral–basic medium (for the *N*-chloroethyl-serine). Starting with this hypothesis, the 2 ketones were analyzed by spectrophotometry, which could prove the existence of these 2 substances as products of the corresponding decomposition reactions. The spectrophotometric study of the β-hydroxypyruvic acid esters (obtained by dissolving β-hydroxypyruvic acid in methanol and ethanol, respectively, and catalyzing the reaction with some drops of HCl) did not show conclusive data of the formation of these hydroxyesters, but the appearance of β-hydroxypyruvic acid in the basified samples (boric–borate buffer, Figure 7) appears to indicate the presence of the previously mentioned hydroxyesters.

Conclusion

The inconveniences of the traditional method for the determination of the decomposition products of chloroamino acids (reaction with 2,4-dinitrophenylhydrazine) has been placed in evidence. An efficient method has been found using HPLC for the quantitative study of the decomposition products of the chloroamino acids applied to the L-serine. The products that were characterized were glycolaldehyde, predominant in neutral–acid medium, and β-hydroxypyruvic acid, predominant in neutral–basic medium; it was possible to identify their yield as a function of the pH and establish their output. With regard to the decomposition of the *N*-chloroesters, it has been verified through HPLC that these products do not form. However, it was possible to identify hydroxyacetone and the methyl esters of β-hydroxypyruvic acid (for the

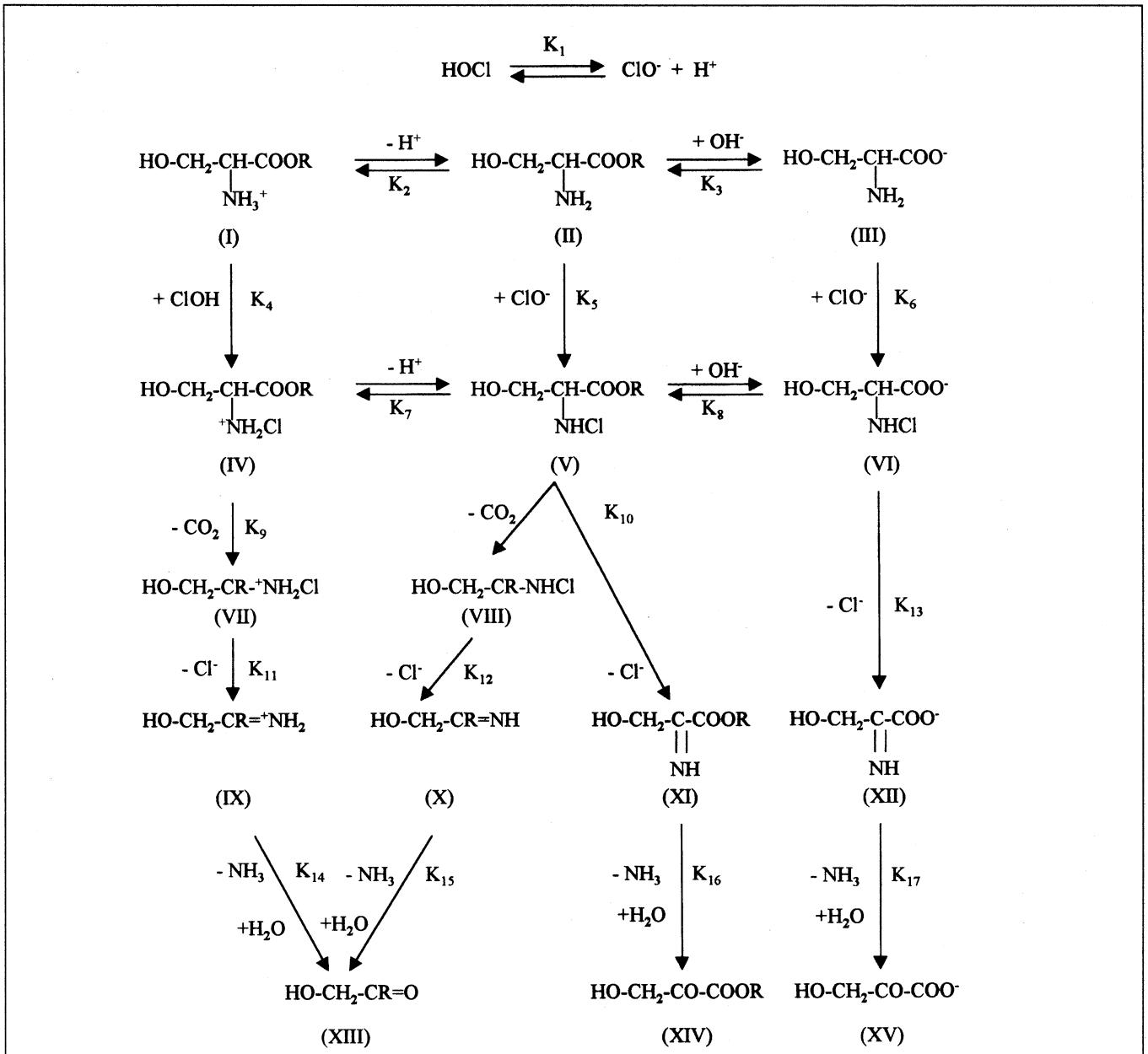

Figure 10. Decomposition mechanism for methyl and ethyl esters of the *N*-chloroserine (R = $-CH_3$, methyl-serine; $-CH_2-CH_3$, ethyl-serine).

N-Chloromethyl-serine) and the 1-hydroxy-2-butanone and the ethyl ester of β-hydroxypyruvic acid (for the *N*-chloroethyl-serine) as degradation products. These results can be explained on the basis of a reaction mechanism (4) made up on the formation of 2 imines. The first one follows a descarboxilative route to provide glycolaldehyde (if the reaction starts from *N*-chloroserine, Figure 9) or the corresponding ketone (if the reaction starts from *N*-chloromethyl-serine or *N*-chloroethyl-serine, Figure 10). The second imide reacts to provide β-hydroxypyruvic acid (if the reaction starts from *N*-chloroserine) or the corresponding esters of the same acid (if the reaction starts from *N*-chloromethyl-serine or *N*-chloroethyl-serine).

Acknowledgments

The authors would like to express their gratitude to the Xunta de Galicia and the Vicerrectorado of Investigation of the University of Vigo for the grants that have permitted this study.

References

1. Y. Levi and J.M. Jestin. Offensive tastes and odors occurring after chlorine addition in water treatment processes. *Water Sci. Technol.* **20:** 269–74 (1988).
2. J.M. Grizzle, S.A. Horowitz, and D.R. Strength. Caged fish as monitors of pollution: effects of chlorinated effluent from a wastewater treatment plant. *Water Resour. Bull.* **24:** 951–59 (1988).
3. W.D. Stambro and W.D. Smith. Kinetics and mechanism of the decomposition of *N*-chloroalanine in aqueous solution. *Environ. Sci. Technol.* **13:** 446–51 (1979).
4. J.M. Antelo, F. Arce, J.G. Fernández, J. Franco, P. Rodríguez, and A. Varela. Studies on the stability of *N*-chloroaminoacids. Decomposition of *N*-chloro-L-serine. *Environ. Technol. Lett.* **9:** 589–98 (1988).
5. J.M. Antelo, F. Arce, J. Franco, F. López, P. Rodríguez, and A. Varela. The kinetics and mechanism of the decomposition of *N*-chloroleucine. *Int. J. Chem. Kinetics* **20:** 433–41 (1988).
6. R. Awad, A. Hussain, and P.A. Crooks. Kinetics and mechanism of decomposition of *N*-chloro-α-amino acids. *J. Chem. Soc.* Perkin Trans. **2:** 1233–38 (1990).
7. J.M. Antelo, F. Arce, J. Crugeiras, J. Franco, F. López, P. Rodríguez, and A. Varela. Estudios sobre la estabilidad de *N*-Cloroaminoácidos. Descomposición de *N*-Cloroprolina y *N*-Clorohidroxiprolina. *Ann. Quim.* **87:** 195–202 (1991).
8. A.J.L. Cooper, J.Z. Ginos, and A. Meister. *Chem. Rev.* **83:** 321 (1983).
9. F.E. Scully, K.E. Mazina, H.P. Ringhand, E.K. Chess, J.A. Campbell, and J.D. Johnson. Identification of organic *N*-chloroamines in vitro in stomach fluid from the rat after chlorination. *Chem. Res. Toxicol.* **3:** 301–306 (1990).
10. M.M. Anderson, S.L. Hazen, F.F. Hsu, and J.W. Heinecke. Human neutrophils employ the myeloperoxidase–hydrogen peroxide–chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. *J. Clin. Invest.* **99:** 424–32 (1997).
11. S.L. Hazen, A. d'Avignon, M.M. Anderson, F.F. Hsu, and J.W. Heinecke. Human neutrophils employ the myeloperoxidase–hydrogen peroxide–chloride system to oxidize α-amino acids to a family of reactive aldehydes. *J. Biol. Chem.* **273:** 4997–5005 (1998).

Manuscript accepted March 30, 1999.