International Journal of Pharmaceutics, 89 (1993) 213–221

IJP 02955

Study of thimerosal degradation mechanism

I. Caraballo, A.M. Rabasco and M. Fernández-Arévalo

Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, Sevilla (Spain)

(Received 29 April 1992)
(Accepted 19 June 1992)

Key words: Thimerosal; Stability; Thiosalicylic acid; Dithiosalicylic acid; Tromethamine

Summary

A specific HPLC method was applied to study the evolution of the concentrations of thimerosal (thiomersal), thiosalicylic acid and 2,2′-di(thiosalicylic) acid over a period of 3 months. The mechanism of degradation of thimerosal in pharmaceutical formulations has been proposed. The protective effect of tromethamine has been also evaluated. It has been found that tromethamine does not exert its effect on the initial reaction of thimerosal decomposition but on the degradation of its initial decomposition products.

Introduction

Thimerosal, ethyl (thiomersal, sodium *o*-mercaptobenzoate) mercury, is an antibacterial agent commonly used as a preservative in pharmaceutical formulations.

It has been reported that thimerosal has stability problems and that some analytical procedures used to quantify this substance (polarography, colorimetry, atomic absorption), are nonspecific when thimerosal is in the presence of its degradation products (Reader and Lines, 1983; Fleitman and Partridge, 1991; Rabasco and Caraballo, 1991).

In previous papers (Caraballo, 1991; Rabasco et al., 1992), we have developed a specific HPLC method to quantify thimerosal in the presence of some of its main decomposition products: thiosalicylic and 2,2′-di(thiosalicylic) acids and the influence of most important formulation factors on the stability of thimerosal has been studied.

In the present paper, the degradation mechanism of thimerosal in different formulations and the protective effect of tromethamine, a compound reported by Doulakas (1988) to improve the stability of thimerosal, are studied.

Materials and Methods

Materials

The following chemicals were used as received: thimerosal (Acofarma, Tarrasa, Spain), thiosalicylic acid (Aldrich-Chemie, Steinheim, Germany), 2,2′-di(thiosalicylic acid) (Sigma Chemical Co., St. Louis, U.S.A.), sodium chloride (Acofarma, Tarrasa, Spain), tromethamine base

Correspondence to: I. Caraballo, Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain.

and hydrochloride (Sigma Chemical Co., St. Louis, U.S.A.), and orthophosphoric acid (Acofarma, Tarrasa, Spain).

The methanol used was HPLC grade (Panreac, Barcelona, Spain) and the water was freshly distilled.

HPLC

The HPLC system consisted of a constant-flow pump (Kontron Instruments, type 420), a Rheodyne type 7125 injector equipped with a 20 μl loop, a variable-wavelength detector (Kontron Instruments, type 432) and an integrator (Konik Instruments, type DataJet 4600). The column used (Brownlee Labs, Spherisorb RP-18, 5 μm particle size, 21 cm × 4.6 mm i.d.) was packed with silica particles bonded with octadecylsilane.

A flow rate of 0.6 ml/min for the mobile phase (methanol : water : phosphoric acid; 65 : 35 : 0.9 v/v) was employed and the variable-wavelength detector was set at 222 nm.

Each peak area was computed automatically by the integrator. Elution was carried out under isocratic conditions at ambient temperature (20 ± 2°C).

Calibration curves

The HPLC method to quantify thimerosal and some of its principal decomposition products (thiosalicylic and dithiosalicylic acids) has been described in our previous papers (Caraballo, 1991; Rabasco et al., 1992).

TABLE 1

Composition and container material of solutions 1–12

Solution no.	NaCl	Tr(B)	Tr(H)	Container
1	0.9			glass
2	0.9	0.007	0.07	glass
3		0.007	0.07	glass
4				glass
5	0.9			polyethylene
6	0.9	0.007	0.07	polyethylene
7		0.007	0.07	polyethylene
8				polyethylene
9	0.9			polypropylene
10	0.9	0.007	0.07	polypropylene
11		0.007	0.07	polypropylene
12				polypropylene

NaCl (% w/v); Tr(B), tromethamine base (% w/v); Tr(H), tromethamine hydrochloride (% w/v).

Preparation of solutions

All solutions were prepared with 20 ppm of thimerosal. Using a cryoscopic osmometer (Gonotec, type Osmomat 030), the contribution of this product to the osmotic pressure of formulations was determined as being insignificant. Therefore, we decided to add sodium chloride and the other substances in the amounts shown in Table 1. These products were placed in their containers and the necessary volume of thimerosal standard solution was added.

The solutions were sampled directly for assay. Determinations were carried out at least in duplicate.

TABLE 2

Calibration curve data of thimerosal

Coefficient of determination 0.9999			Estimated constant term $-2.4E-4$		
Multiple correlation coefficient 0.9999			Standard error of estimate 0.0088		
Source	D.F.	Sum. of squares	Mean of squares	*F*	Probability
Regression	1	27.9115	27.9115	351757	0.0000
Residuals	10	$7.934E-4$	$7.934E-5$		
Total	11	27.9123			
Regression coefficient		standard coefficient	standard error	*T*	probability
221.985		0.9999	0.3742	593.091	0.0000

Results and Discussion

Calibration curves

A plot of peak areas vs concentrations was linear in the ranges of 5–200, 0.15–10 and 0.1–5 ppm of thimerosal, thiosalicylic and dithiosalicylic acids, respectively. Regression analysis of the calibration curves gave the statistical parameters shown in Tables 2–4.

The specificity of the method is illustrated in Fig. 1, where complete separation was observed for the three compounds studied. The retention times for thiosalicylic acid, thimerosal and dithiosalicylic acid were 5.8, 7.6 and 9.8 min, respectively.

To assess the reproducibility of this method, four replicate samples from a solution containing thimerosal (20 ppm) were analysed on each of five different days. The coefficients of variation were 1.44 (apparatus), 1.13 (within-day) and 1.72 (between days).

Study of the degradation process

The process of degradation of thimerosal in ophthalmic solutions has not been studied in detail. The general process has been reported by Reader and Lines (1983). The latter authors suggest the reaction to be as shown in Fig. 2.

In the work of Doulakas (1988), it was found that tromethamine increases the stability of thimerosal in aqueous solution and can be used as a protectant in thimerosal formulations. Hence, we have included in our study solutions containing tromethamine (solutions 2,3,5,6,10,11).

In previous papers (Caraballo, 1991; Rabasco et al., 1992) we found that tromethamine (base or hydrochloride) increased the stability of thimerosal in solutions containing sodium chloride. This effect was similar when we added tromethamine base or a tromethamine base and hydrochloride mixture. However, even in the presence of tromethamine, thimerosal undergoes extensive degradation. Moreover, considering so-

TABLE 3

Calibration curve data of thiosalicylic acid

Coefficient of determination 0.9990			Estimated constant term −0.0085		
Multiple correlation coefficient 0.9995			Standard error of estimate 0.0064		
Source	D.F.	Sum. of squares	Mean of squares	*F*	Probability
Regression	1	0.80391	0.80391	19855.6	0.0000
Residuals	20	$8.097E-4$	$4.048E-5$		
Total	21	0.80472			
Regression coefficient		standard coefficient	standard error	*T*	probability
0.07096		0.9995	$5.0362E-4$	140.910	0.0000

TABLE 4

Calibration curve data of dithiosalicylic acid

Coefficient of determination 0.9994			Estimated constant term: $-6.61E-4$		
Multiple correlation coefficient 0.9997			Standard error of estimate: 0.0021		
Source	D.F.	Sum. of squares	Mean of squares	*F*	Probability
Regression	1	0.07401	0.07401	16879.1	0.0000
Residuals	10	$4.384E-5$	$4.384E-6$		
Total	11	0.07405			
Regression coefficient		standard coefficient	standard error	*T*	probability
0.04534		0.9997	$3.4897E-4$	129.920	0.0000

lutions without sodium chloride, tromethamine addition has no significant protective effect. In the previous investigation, we also studied the influence of the container material (polyethylene, polypropylene and glass). We observed that solutions containing sodium chloride and thromethamine exhibited very similar behaviour on storage in the three different types of containers, without any statistically significant difference. On the other hand, formulations containing sodium chloride without tromethamine undergo more extensive degradation when stored in plastic containers as compared with glass containers.

In the present study, and in order to study the decomposition process of thimerosal, the concentrations of thimerosal and thiosalicylic and dithiosalicylic acids were evaluated for 3 months.

Fig. 3 depicts the thimerosal concentration profiles in glass, polyethylene and polypropylene containers.

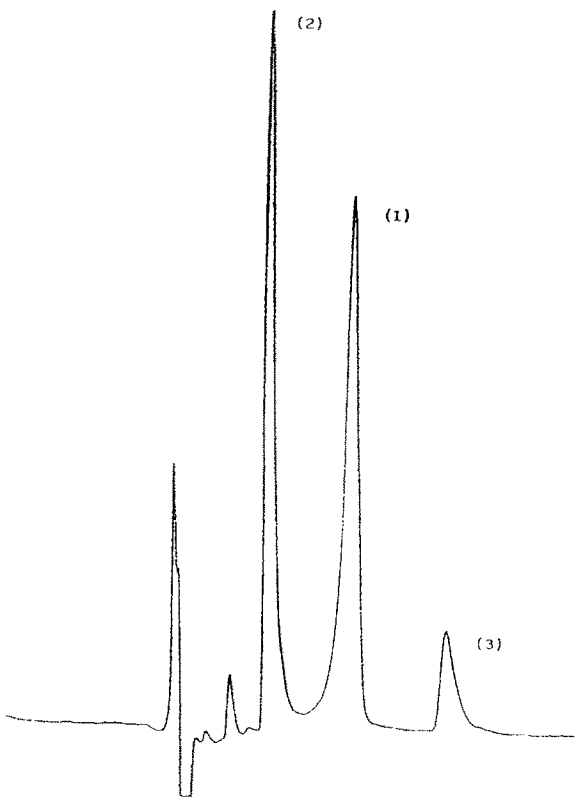


Fig. 1. Chromatogram showing the peaks corresponding to thimerosal (1), thiosalicylic acid (2) and dithiosalicylic acid (3).

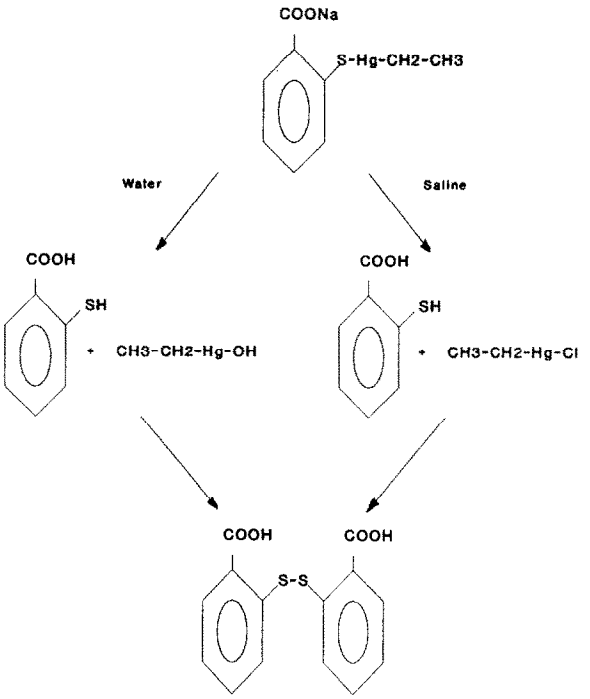


Fig. 2. Reaction scheme for the decomposition of thimerosal proposed by Reader and Lines (1983).

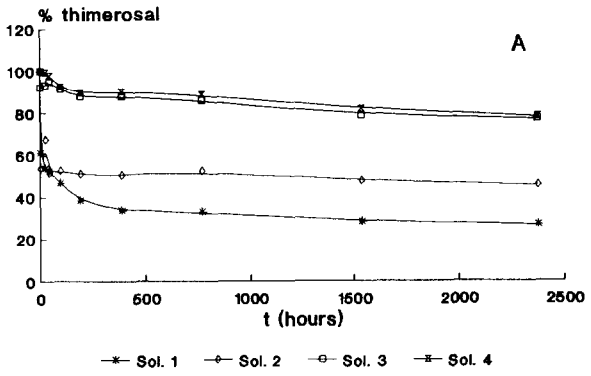
The first step in our study was to fit the data of thimerosal concentrations vs time to zero, first, second and third order kinetics, using non-linear regression programs. Data from all the regressions applied were very poor. By representing $1/[(n-1)\cdot C^{n-1}]$ vs time ($n = 0$–3), the correlation coefficients were also too low. Therefore, we cannot confirm the reaction order of thimerosal degradation as the AIC and the correlation coefficient values were too similar for all the kinetic processes studied.

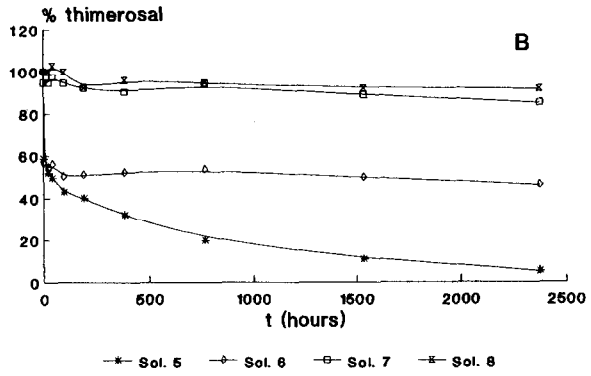
From our data, and the knowledge that several degradation products of thimerosal exist, we believe that the decomposition process of this drug progresses through multiple reactions, which result in a complex kinetic process. In Fig. 3, one can observe different kinetic behaviour between thimerosal solutions with and without sodium chloride. For this reason, the two different types of solutions were studied separately.

Solutions of thimerosal with sodium chloride

In Fig. 4 we show in detail the initial behaviour of thimerosal. As can be observed, the

first obstacle that we have found to explain the degradation kinetics of thimerosal as a single reaction is the great initial and almost instantaneous decrease in its concentration, followed by a sudden decrease in the rate of decomposition. This situation led us to believe that the decomposition of thimerosal to produce ethylmercuric chloride and thiosalicylic acid is a reversible reaction. We have demonstrated this fact by preparing saline solutions of thimerosal supplemented with thiosalicylic acid and observing how these solutions underwent lower degradation than those prepared without this last product.





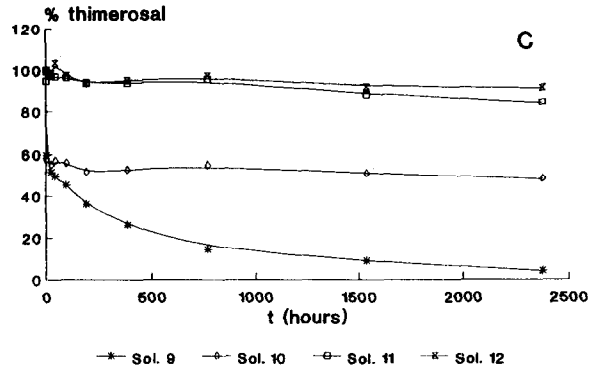


Fig. 3. Thimerosal concentration profiles in glass (A), polyethylene (B) and polypropylene (C) containers.

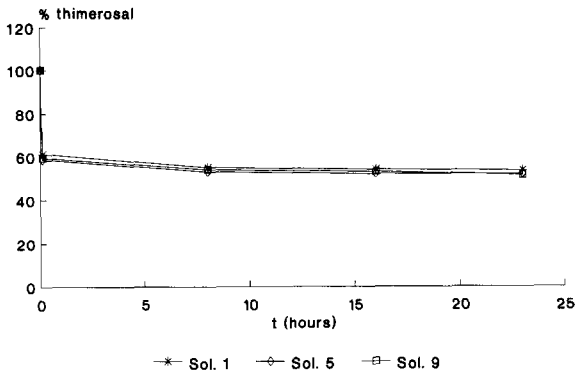


Fig. 4. Initial decrease of thimerosal concentration in solutions containing sodium chloride.

For continuing the study, and as we have carried out an analytical method to quantify thimerosal and both of its principal decomposition products, we have measured their concentrations. The results obtained from 0.9% saline solutions with and without tromethamine, are shown in Figs 5–7.

The profiles of the solutions containing tromethamine allowed us to elucidate the mechanism of the studied reaction.

Based on the reversibility of the reaction to produce thiosalycilic acid and ethylmercuric chloride from thimerosal, and considering our experimental data (Figs 5–7), we propose the following mechanism of degradation of thimerosal in solutions containing sodium chloride:

The entire process should be divided in two basic stages. The first would be the next reversible reaction:

$$\text{thimerosal} \underset{(-1)}{\overset{(1)}{\rightleftharpoons}} \text{thiosalicylic acid} + \text{ethylmercuric chloride}$$

Reaction (1) is very fast in the presence of chloride anions and a rapid decrease in thimerosal concentrations will result, as reported in previous papers (Pohloudek-Fabini and Martin, 1981; Reader and Lines, 1983; Thoma and Schubert, 1987). Consequently, the thiosalicylic acid and ethylmercuric chloride concentrations will rise suddenly. This situation is in agreement with the concentration profiles in our study (Figs 5–7).

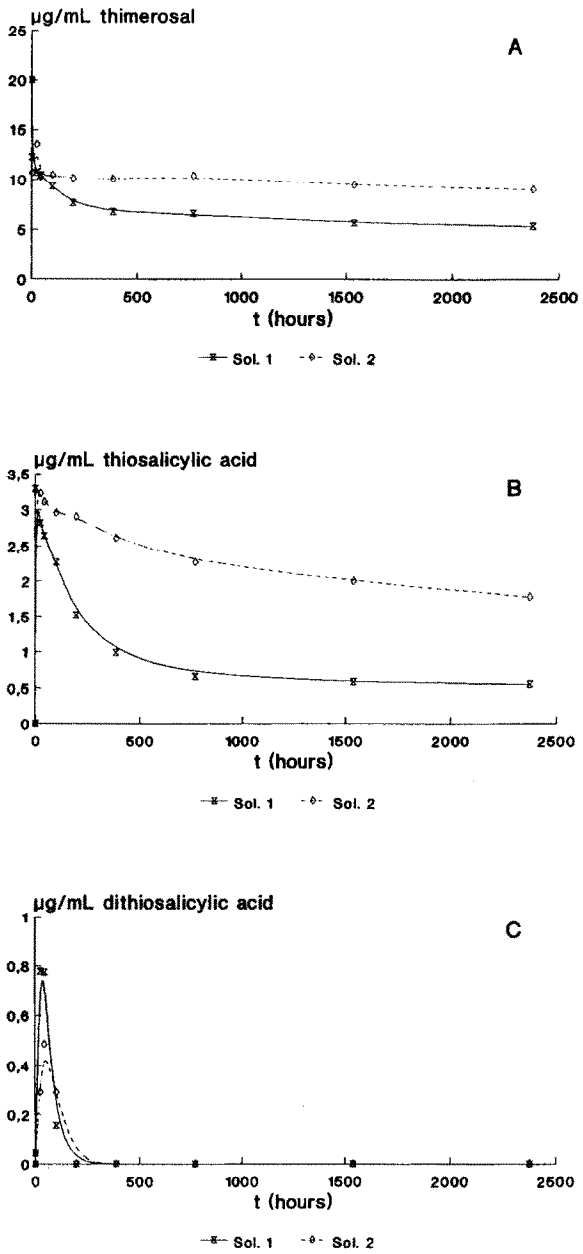


Fig. 5. Concentration profiles of thimerosal (A), thiosalicylic acid (B) and dithiosalicylic acid (C) of solutions with (— — —) and without (———) tromethamine. The solutions contained sodium chloride and were stored in glass containers.

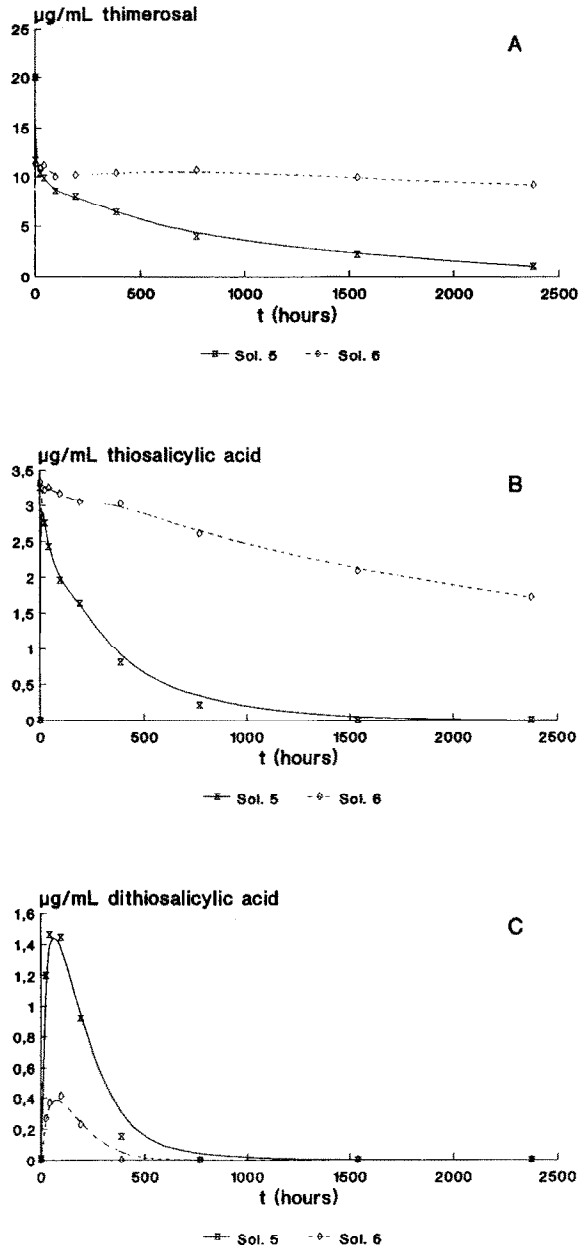


Fig. 6. Concentration profiles of thimerosal (A), thiosalicylic acid (B) and dithiosalicylic acid (C) of solutions with (— — —) and without (———) tromethamine. The solutions contained sodium chloride and were stored in polyethylene containers.

The second stage would be concomitant with the first one and involves the oxidation of thiosalicylic acid to produce dithiosalicylic acid with the corresponding reduction of ethylmercuric chloride to metallic mercury, consistent with the data reported by Reader and Lines (1983). This stage would be continued with the decrease in dithiosalicylic acid concentration, as illustrated in panel C of Figs 5–7. The decomposition of this last product yields substances with greater hydrophilicity. This fact is supported by HPLC, as shown in Fig. 8. As a sort of a summary, the second stage is represented in the following scheme:

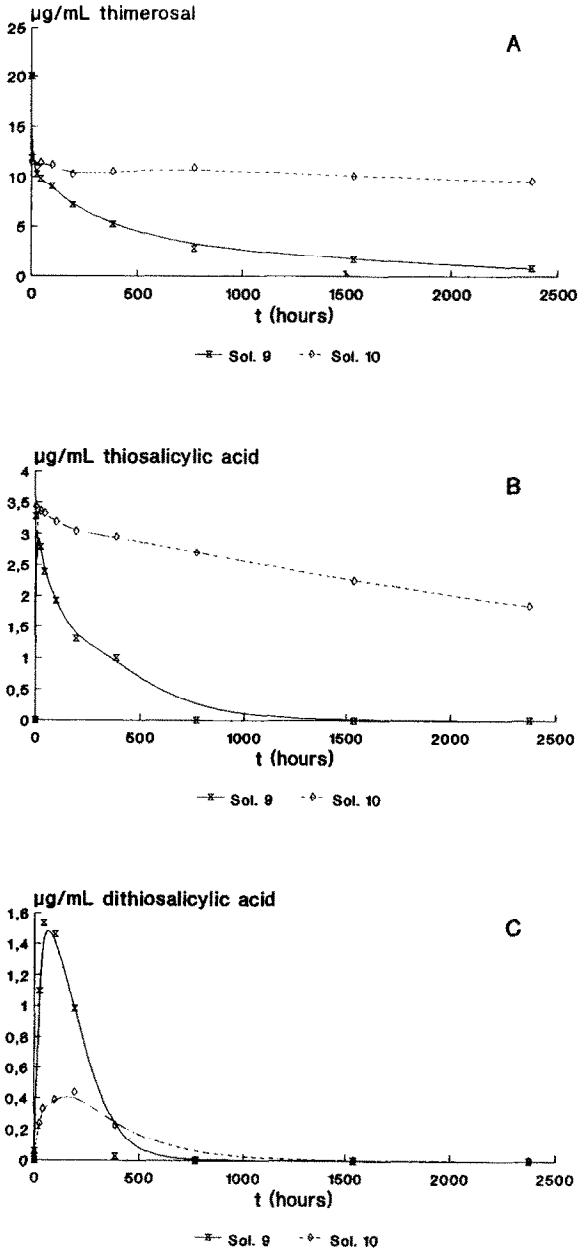


Fig. 7. Concentration profiles of thimerosal (A), thiosalicylic acid (B) and dithiosalicylic acid (C) of solutions with (— — —) and without (———) tromethamine. The solutions contained sodium chloride and were stored in polypropylene containers.

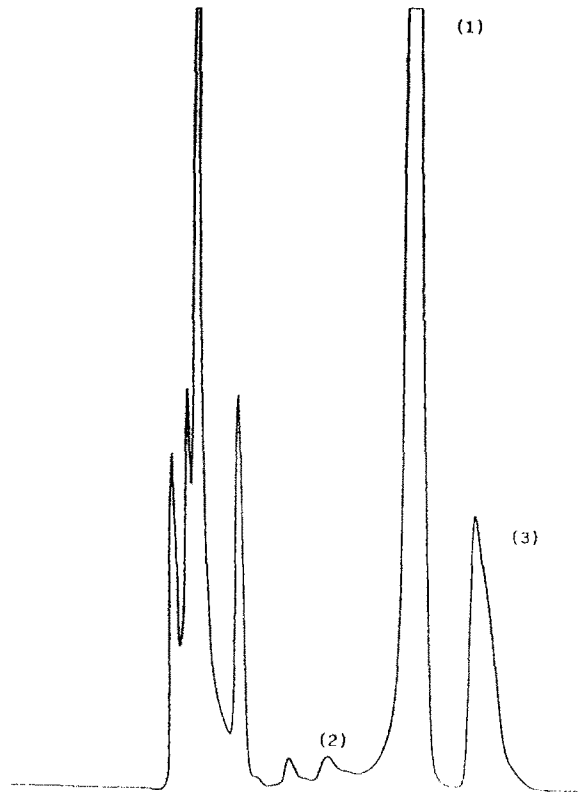


Fig. 8. Chromatogram of a degraded thimerosal solution. The peaks corresponding to the new decomposition products appear near the solvent front (1, thimerosal; 2, thiosalicylic acid; 3, dithiosalicylic acid).

thiosalicylic acid + ethylmercuric chloride

$\xrightarrow{(2)}$ dithiosalicylic acid + Hg^0

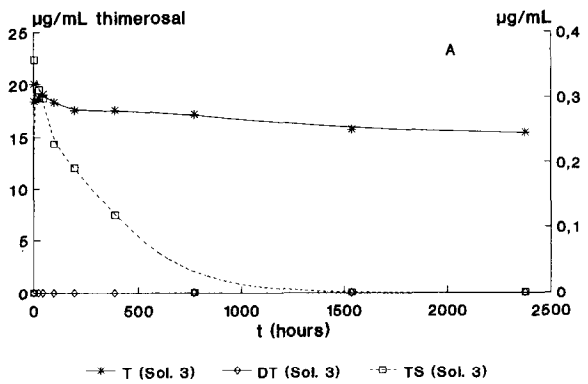
$\downarrow$ (3)

decomposition products

The reactions of the second stage prevent the reversible process of the first stage reaching the equilibrium state and induce the later degradation of thimerosal after its initial decrease.

The oxidation of thiosalicylic to dithiosalicylic acid is an irreversible reaction (under our experimental conditions), as has been confirmed by HPLC. Furthermore, reaction (2) should proceed at a slower rate than reaction (1) and should be the rate-determining step in the degradation thimerosal in saline solutions, once the initial diminution in its concentration has occurred.

As shown in panel A of Figs 5–7, tromethamine addition has no effect on the degradation rate of thimerosal in stage 1 (the initial decrease in thimerosal concentration is similar in solutions



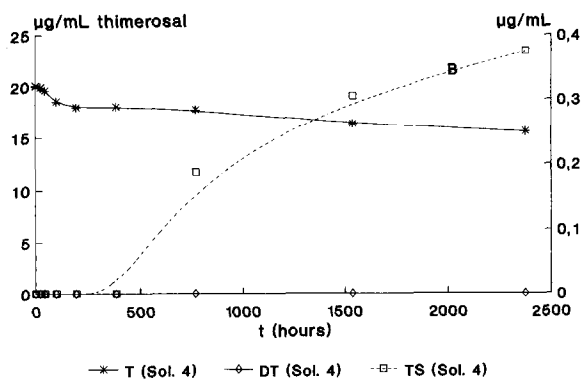
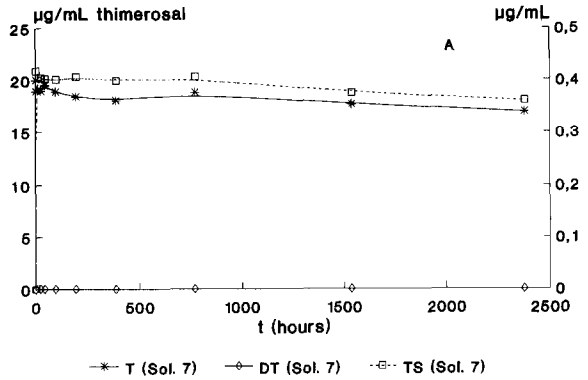


Fig. 9. Concentration profiles of thimerosal (T), thiosalicylic acid (TS) and dithiosalicylic acid (DT) of solutions with (Sol. 3) and without (Sol. 4) tromethamine, stored in glass containers (scale: [T], 0–25 μg/ml; [TS] and [DT], 0–0.4 μg/ml).



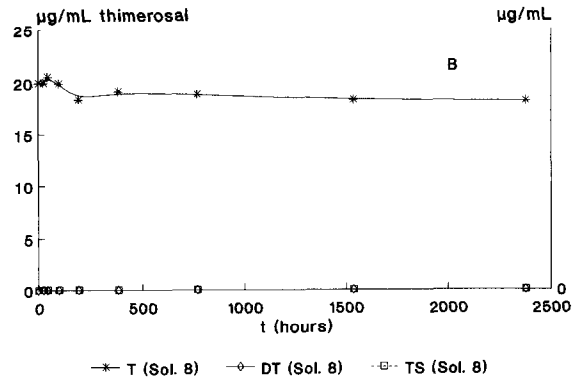


Fig. 10. Concentration profiles of thiomerosal (T), thiosalicylic acid (TS) and dithiosalicylic acid (DT) of solutions with (Sol. 7) and without (Sol. 8) tromethamine, stored in polyethylene containers (scale: [T], 0–25 μg/ml; [TS] and [DT], 0–0.5 μg/ml).

with and without tromethamine). In view of these results, we can conclude that tromethamine exerts its protective effect in reaction (2) and that this reaction controls the second stage of the process of thimerosal degradation. Furthermore, this shows that the decomposition of thimerosal to produce thiosalicylic acid and ethylmercuric chloride is a reversible process. For this reason, higher thiosalicylic acid concentrations in solutions containing tromethamine (see panel B of Figs 5–7) result in a lower extent of thimerosal degradation during the second stage of this process.

As demonstrated in panel C of Figs 5–7, the concentration of dithiosalicylic acid decreased af-

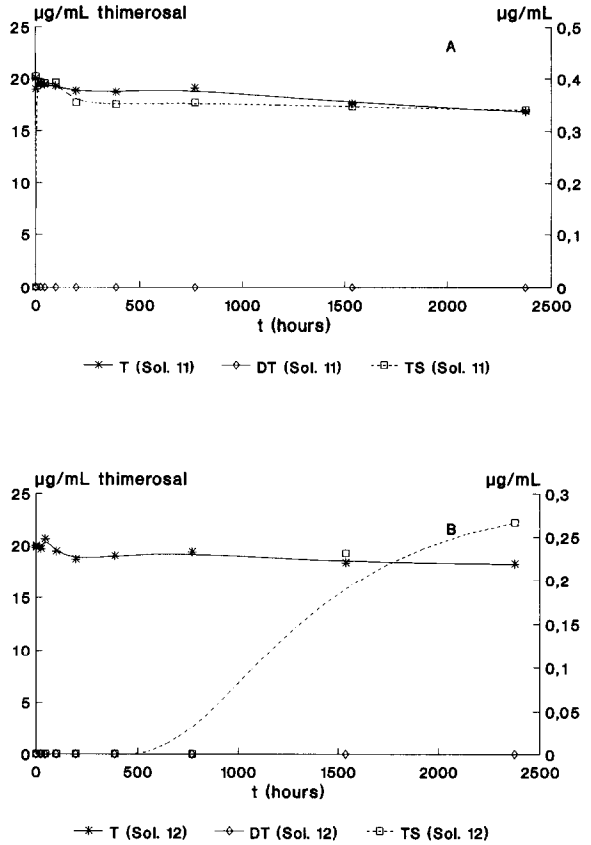


Fig. 11. Concentration profiles of thimerosal (T), thiosalicylic acid (TS) and dithiosalicylic acid (DT) of solutions with (Sol. 11) and without (Sol. 12) tromethamine, stored in polypropylene containers (scale: [T], 0–25 μg/ml; [TS] and [DT], 0–0.5 μg/ml.

ter 5 days of storage until it fell below our analytical threshold.

Solutions of thimerosal without sodium chloride

In these formulations, we observed a smaller extent of thimerosal degradation in comparison with saline solutions (Fig. 3). Therefore, the concentrations of thiosalicylic and dithiosalicylic acids are insufficient to allow us to verify the degradation mechanism previously proposed. This is illustrated in Figs 9–11. Nevertheless, there are no reasons to reject a similar mechanism with two unique exceptions: in these solutions, the thimerosal decomposition reaction (reaction (1)) is much slower than in those with sodium chloride. Moreover, ethylmercuric hydroxide would be found instead of ethylmercuric chloride. The reaction would be:

$$\text{thimerosal} \underset{(-1)}{\overset{(1)}{\rightleftharpoons}} \text{thiosalicylic acid}$$

$$+ \text{ethylmercuric hydroxide} \xrightarrow{(2)} \text{dithiosalicylic acid} + Hg^0$$

$$\downarrow (3)$$

$$\text{decomposition products}$$

This model also explains the fact that tromethamine does not produce any positive influence on thimerosal in these type of solutions, since in these, the reaction that exerts the kinetic control over the entire decomposition process is not reaction (2) but reaction (1).

The proposed reaction mechanism for the decomposition of thimerosal, provides a reasonable explanation for the kinetic data obtained for all the thimerosal formulations studied.

References

Caraballo, I., Estudio de la cinética de degradación del tiomersal en preparados farmacéuticos, Tesis de Licenciatura, Universidad de Sevilla (1991).

Doulakas, J., *Ger. Offen.* DE 3.612.538 (through *Chem. Abstr.*, 108 (1988) 368).

Fleitman, J.S. and Partridge, I.W., Thimerosal analysis in ketorolac tromethamine ophthalmic solution. Comparing HPLC and colorimetric techniques. *Drug Dev. Ind. Pharm.*, 17 (1991) 519–530.

Pohloudek-Fabini, R. and Martin, E, Einfluß der Gaspermeabilität von Kunststoffen auf die Stabilität des Thiomersals. *Pharmazie*, 36 (1981) 683–685.

Rabasco, A.M. and Caraballo, I., Determinación por HPLC del tiomersal en preparados farmacéuticos, *VIII Jornadas Farmacéuticas Españolas*, Sevilla, 3–6 December 1991.

Rabasco, A.M., Caraballo, I. and Fernández-Arévalo, M., Formulation factors affecting thimerosal stability. submitted.

Reader, M.J. and Lines, C.B., Decomposition of thimerosal in aqueous solution and its determination by high-performance liquid chromatography. *J. Pharm. Sci.*, 72 (1983) 1406–1409.

Thoma, K. and Schubert, O.E., Thiomersal. Wie chemisch instabil ist dieses Konservierungsmittel? *Dtsch. Apoth. Ztg.*, 127 (1987) 1867–1869.