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THE EFFECT OF DISODIUM EDETATE AND SODIUM CHLORIDE ON THE STABILITY OF ETHYLMERCURY ARISING FROM THE DECOMPOSITION OF THIMEROSAL (THIOMERSAL)

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ABSTRACT

The effect of disodium edetate and sodium chloride on the stability of the ethylmercuric ion arising from the decomposition of thiomerosal has been investigated using species-specific chromatographic assays. Disodium edetate stabilises both the ethylmercuric ion and thimerosal whereas chloride stabilises the ethylmercuric ion but promotes decomposition of the thimerosal.

INTRODUCTION

Thimerosal (thiomersal) (TM), an antimicrobial preservative used in pharmaceutical systems, is the sodium salt of the complex formed between thiosalicylic acid (TSA) and the ethylmercuric (EtHg) ion. The preservative is unstable to light in aqueous solution and decomposes to 2,2-dithiosalicylic acid (DTSA), TSA and the EtHg ion (1-8).

Methods of analysis include atomic absorption spectroscopy (6,9), polarography (10), colorimetry (11-13) and a variety of methods employing

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high-performance liquid chromatography (HPLC) (2-8,13-20). Most of these chromatographic methods measure intact TM and as a consequence give significantly different results when applied to degraded samples to the methods which measure total mercury such as atomic absorption spectroscopy (6,9) and colorimetry (2,13,15).

Recently HPLC methods have been developed which quantitate total available EtHg either as a dithiocarbamate complex (4,6) or, by the addition of excess TSA prior to chromatography, as TM(5).

Chemically degraded TM has been shown to have equivalent or greater antimicrobial activity to undegraded samples (13,21) and it is reasonable to suppose that, as with other organo-mercurials, the activity resides in the EtHg ligand (22).

This paper reports the results of a series of investigations on the influence of sodium chloride and disodium edetate (EDTA) on the available EtHg in TM solutions.

METHODS

Materials and Reagents

TM and TSA (Sigma, St. Louis, MO, USA) were used as supplied. All other chemicals were analytical or HPLC grade.

Chromatographic Methods

TM content was measured by the HPLC method of Lam et al. (16) and total EtHg by HPLC of the corresponding piperidinedithiocarbamate complex according to the method of Parkin (4) and by the adaptation of the method of Lam et al (16) involving addition of excess TSA to the sample of TM to be quantitated (5). The chromatographic conditions and equipment were the same as those reported earlier from this laboratory for these assays (4,5).

Thermal Decomposition of TM

Solutions of TM (0.01% w/v) in water, pH 5 acetate buffer (0.05M) and pH7 phosphate buffer (0.05 M) with and without disodium edetate (0.05% w/v) were prepared, transferred immediately to 10 mL ampoules and submitted to autoclaving at 121°C for 15 min. The resulting solutions were submitted to

TABLE 1

Percent Thimerosal remaining Following Autoclaving at 121°C for 15 min.

	TM	Total EtHg by dithiocarbamate method (4)	Total EtHg by method utilising excess TSA(5)
Without Disodium Edetate			
Water	90.1	90.3	89.2
pH 5 Acetate Buffer	41.5	91.5	91.5
pH 7 Phosphate Buffer	22.5	90.1	91.4
With Disodium Edetate			
Water	92.6	100.6	99.1
pH 5 Acetate Buffer	95.7	98.8	98.0
pH 7 Phosphate Buffer	94.8	98.9	96.7

chromatographic analysis. The solutions were protected from light and all operations performed in subdued lighting and the experiments were performed in duplicate.

Storage of TM Solution in LDPE Bottles

Solutions of TM (0.01% w/v) were prepared in water and pH 5.5, 6.5 and 7.5 phosphate buffers (0.05 M) with and without sodium chloride (0.9% w/v). These were transferred to 15 mL LDPE eye-dropper bottles and stored in the dark at 20 ± 2°C and assayed at regular intervals by the HPLC method of Parkin (5). The studies were performed in triplicate.

RESULTS AND DISCUSSION

When solutions of the preservative were submitted to thermal degradation (121°C for 15 min.) in the absence of light there was a substantial loss of TM in solutions without disodium edetate (10-80% loss) (Table 1). However there was less than 10% loss of EtHg when the samples were submitted to analysis by both methods (4,5) which have been shown to be specific for the EtHg ligand in the presence of TM (Table 1). When this experiment was repeated in the presence of disodium edetate there were only small losses under all conditions of the EtHg

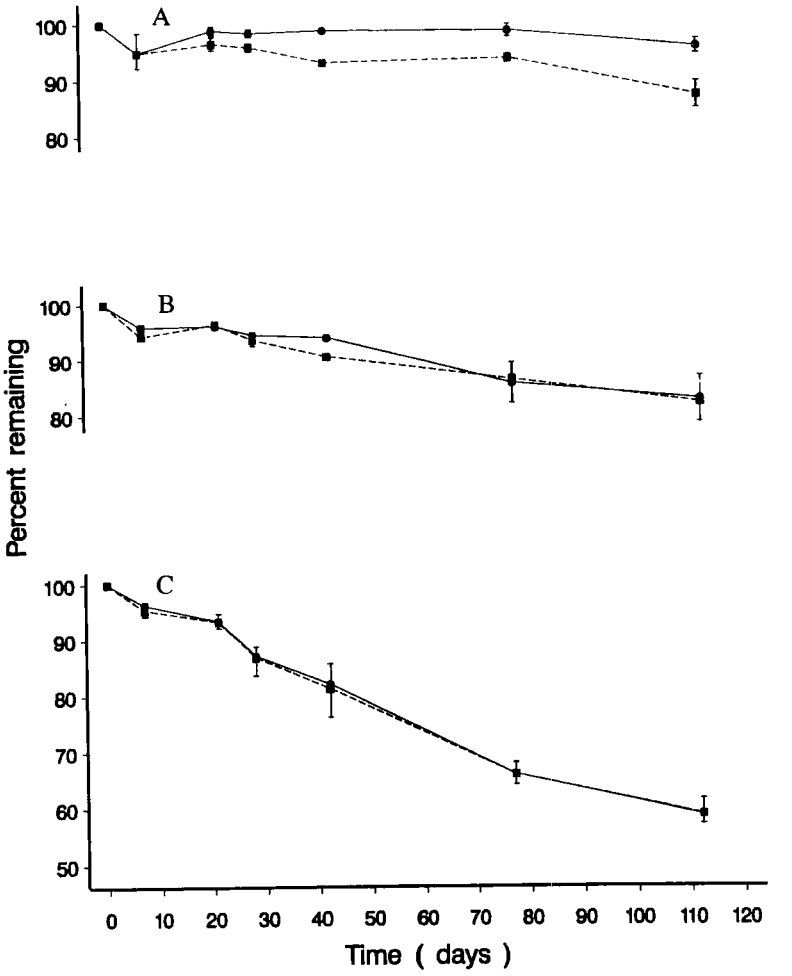

Fig. 1: Loss of thiomersal ----■---- and total available ethylmercury ——●—— when thiomersal was stored in LDPE dropper bottles in A - water, B - pH 6.5 phosphate buffer and C - 7.5 phosphate buffer(± 1 SD, n = 3).

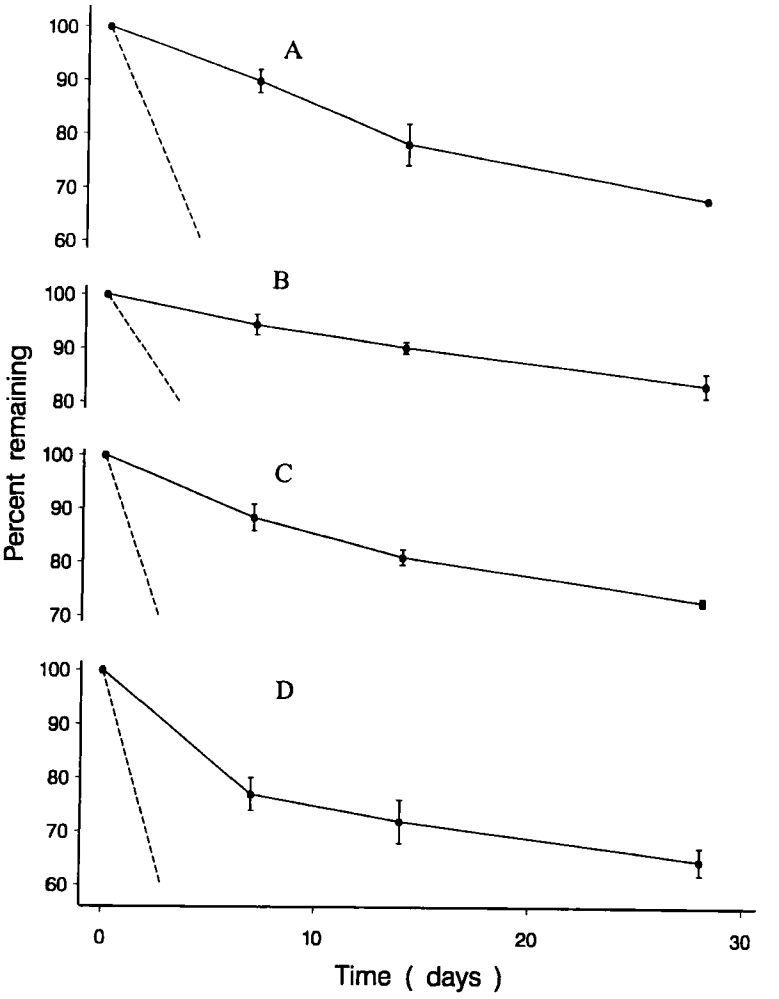

Fig 2: Loss of thiomersal ------ and total available ethylmercury —•— when thiomersal was stored in LDPE dropper bottles in A: 0.9% w/v sodium chloride, B: pH 7.5 phosphate buffer containing 0.9% w/v sodium chloride, C: pH 6.5 phosphate buffer containing 0.9% w/v sodium chloride, and D: pH 5.5 phosphate buffer containing 0.9% w/v sodium chloride. (±1 SD, N=3).

ligand (Table 1). Under all conditions the concentration of total available EtHg was equal to or greater than the TM as would be expected and the loss of ETHg in the presence of disodium edetate was insignificant. The presence of the complexing agent in the solution stabilises both the decomposition of TM and the EtHg.

Studies were also undertaken measuring both TM and total EtHg upon storage of solutions of TM in buffered solutions in the presence and absence of sodium chloride in LDPE eye-dropper bottles. Under these conditions there were substantial losses of TM and EtHg the losses being greatest at the highest pH (Fig. 1A-C). In the presence of sodium chloride losses of TM were rapid as has been reported previously but little effect is noted on loss of the EtHg species (Fig. 2A-D).

The chemistry of TM in solution is very complex and minor changes in formulation may profoundly influence the stability (2-4,6-8). Of greater significance than that of TM is the stability of the EtHg species formed during the degradation as this is probably the active antimicrobial species (22) and this would explain the maintenance of antimicrobial activity in highly degraded samples of TM (13,21). The complexation chemistry of EtHg has never been investigated in detail but it would be expected to be very similar to that of the homologous methylmercury which has been widely studied due to its significance in the environmental toxicology of mercury (23,24).

The loss of TSA by oxidation to DTSA would free EtHg which would complex with the water (as $CH_3CH_2Hg^+$, $(CH_3CH_2Hg)_2OH^+$ or CH_3CH_2HgOH dependent upon the pH) (25) or other buffer components (22,23). This would explain the bewildering variability in the stability of TM as influenced by pH, buffer components and other compounds present (3,7,8,13) as the oxidation of the TSA would be expected to be suppressed by complexation to the EtHg.

The level of EtHg species present in degraded ophthalmic formulations containing TM correlates well with the level of mercury present measured by atomic absorption spectroscopy (6) indicating that there are no other soluble mercury-containing species arising from degradation and therefore the measurement of EtHg by the reported chromatographic procedures (4-6) may afford an accurate chemical assessment of available antimicrobial species present.

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