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High-performance liquid chromatographic investigation of the interaction of phenylmercuric nitrate and sodium metabisulphite in eye drop formulations

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ABSTRACT

The degradation of phenylmercuric nitrate in the presence of sodium metabisulphite in eye drop formulations has been investigated using a stability-indicating high-performance liquid chromatographic (HPLC) method. HPLC methods have been developed for the quantitation of the principal degradation products (diphenylmercury, benzenesulphonic acid and benzenesulphinic acid) and a mechanism is proposed for their formation. The pharmaceutical significance of the interaction is briefly discussed.

INTRODUCTION

A number of studies have shown that when sodium metabisulphite is added as an antioxidant to ophthalmic products containing phenylmercuric (PM) salts, a loss of PM salts occurs when the product is routinely heat-sterilised[1–6]. These studies afforded conflicting results when products were assessed for antibacterial activity by microbiological procedures, but in all studies where PM salt concentrations were determined by atomic absorption spectrophotometry (AAS)[1,2,5,6] and high-performance liquid chromatography (HPLC)[5], losses of 75–100% PM salt were reported. An exception was the study of Richards *et al.*[2], who quantitated the PM nitrate by AAS using both an air–acetylene technique and a cold-vapour method and found that there was an 80% loss when determined by the air–acetylene technique but mercury levels remained unchanged when the cold-vapour method was employed. They concluded that a complex was formed between the metabisulphite and the PM nitrate during autoclaving which is more refractory in the air–acetylene flame and more difficult to reduce to elemental mercury than aqueous PM nitrate solutions and that this influenced the analytical results.

Despite these observations, the British Pharmacopoeia (1980)[7] contained a number of ophthalmic monographs which incorporated both PM nitrate and sodium

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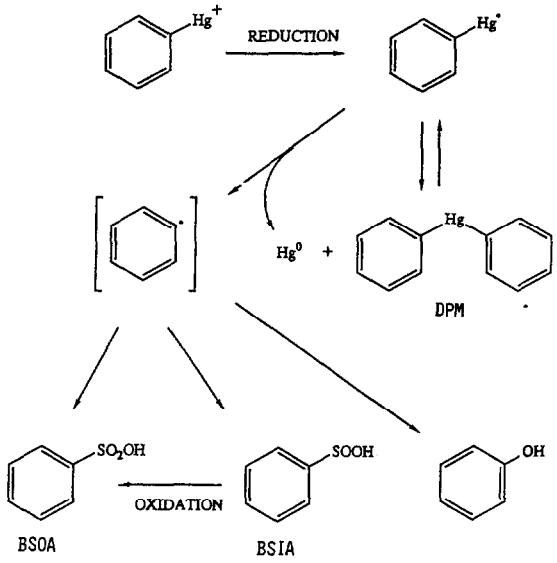

Fig. 1. Proposed route for the degradation of the PM ion in the presence of sodium metabisulphite.

metabisulphite. The current British Pharmacopoeia (1988)[8], in its general monograph on eye drops, contains the statement that "care should be taken to ensure compatability between the antioxidant and the antimicrobial preservative" but does not specifically preclude products containing both these materials; the current Australian Pharmaceutical Formulary and Handbook (1988)[9] lists several ophthalmic products which contain both PM nitrate and sodium metabisulphite.

N-Disubstituted dithiocarbamate complexes have been used to chromatograph and quantitate organomercury and inorganic mercury[10] and the author[10,11] has developed an HPLC assay using these reagents for application to ophthalmic products.

This paper reports an investigation using HPLC of the decomposition following heat-sterilisation of PM nitrate by sodium metabisulphite in simple solution and amethocaine eye drops[9]. The time-course of this decomposition at 100°C is followed with quantitation of PM nitrate and diphenylmercury (DPM), a transient intermediate formed during the degradation. The terminal degradation products following autoclaving at 121°C have also been identified and quantitated and were found to be principally elemental mercury, benzenesulphonic acid (BSOA) and benzenesulphinic acid (BSIA). A mechanism is proposed for the formation of these products (Fig. 1).

EXPERIMENTAL

Materials

PM nitrate (BDH, Poole, U.K.), DPM (Fluka, F.R.G.), amethocaine hydrochloride, BSOA and BSIA as the sodium salt (Sigma, St. Louis, MO, U.S.A.) were used in this study. All other chemicals were analytical-reagent grade.

The morpholine salt of morpholinedithiocarbamate (MDTC) and diethylamine

salt of diethylaminedithiocarbamate (DEADTC) were synthesized as reported previously[11,12]. The MDTC and DEADTC complexing reagents were prepared by dissolving 60 mg of the salts in 75% aqueous acetonitrile for MDTC and acetonitrile for DEADTC (100 ml).

Chromatographic equipment

A liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.), equipped with a 501 pump, 712 WISP injector, 490 variable-wavelength detector and HP 3396A integrator, together with a column of octadecylsilica (Waters Assoc.) 30 cm × 3.9 mm I.D., 10 μm particle size, was used for analysis of the degradation products and of PM nitrate. Injection volumes of 20 μl were used unless otherwise specified.

Spectrophotometric equipment

UV spectra were obtained using an HP 8450 UV–VIS spectrophotometer (Hewlett-Packard, PA, U.S.A.).

Chromatographic procedures

PM and mercury salts were determined by the addition of 1 ml of DEADTC reagent to 1 ml of sample and the resulting complexes submitted to chromatographic analysis using $1 \cdot 10^{-4}$ *M* disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) in acetonitrile–water (75:25) at a flow-rate of 1.8 ml min^{-1} and monitoring at 258 nm.

DPM in the presence of PM nitrate was determined by the addition of 1 ml of MDTC reagent to 1 ml of sample to complex the PM ion followed by chromatography using $1 \cdot 10^{-4}$ *M* Na_2EDTA in acetonitrile–water (65:35) at a flow-rate of 1.8 ml min^{-1} and monitoring at 225 nm.

BSOA and BSIA were analysed by chromatography using methanol–water (33:67) containing 0.1% (w/v) tetrabutylammonium hydrogensulphate and monitoring at 220 nm.

Phenol was analysed using a mobile phase of methanol–water (40:60) at a flow-rate of 1.5 ml min^{-1} with monitoring at 215 nm.

Kinetic studies

These were performed by adding 200 mg sodium metabisulphite to a solution of PM nitrate (200 ml of $2 \cdot 10^{-4}$ *M*) or amethocaine eye drops APF[9] [200 ml, modified by containing $2 \cdot 10^{-4}$ *M* PM nitrate, equivalent to 0.00634% (w/v) PM nitrate] in a flask with condenser in a boiling water bath. The reaction was equilibrated to temperature prior to the addition of sodium metabisulphite and the solution was kept under nitrogen for the course of the study. Samples of 5 ml were withdrawn at regular intervals, transferred to a vial, cooled on ice and centrifuged for 10 min using a refrigerated centrifuge, and the supernatants were then submitted to HPLC analysis by the chromatographic procedures for PM nitrate and DPM.

Terminal degradation products

Aliquots (10 ml) of the solutions used in the kinetic studies were transferred to 20-ml glass ampoules and autoclaved for 2 h at 121°C. The resulting solutions were transferred to tubes and centrifuged for 30 min. The supernatants were transferred to

second tubes. The residues, which were not visible, were heated at 40°C for 15 min with eight drops of concentrated nitric acid–water (1:1), water was added to 7 ml, saturated sodium acetate was added dropwise to adjust the pH to 4–5 and the solution was made to 10 ml and submitted for analysis for Hg^{II}.

Solutions of PM nitrate (5 ml of $2.5 \cdot 10^{-4}$ *M*) containing sodium metabisulphite (0.2%, w/v) and other materials as specified were sealed in 10-ml glass ampoules and autoclaved for 1.5 h at 121°C. The contents of the ampoules were assayed in the following manner.

(a) For water-soluble inorganic mercury salts and residual PM nitrate following centrifugation for 10 min.

(b) Elemental mercury and insoluble mercury salts were quantitated by the addition of nitric acid (2 ml) to the opened ampoule and digestion at 50°C for 15 min to dissolve the elemental mercury. The cooled solutions were transferred quantitatively to 50-ml volumetric flasks, neutralised to pH 4–5 with saturated sodium acetate solution, diluted to volume with water and submitted to analysis.

(c) Phenol was quantitated following centrifugation of the contents of an ampoule for 10 min.

(d) BSOA and BSIA were analysed by centrifugation of the contents of an ampoule to remove insoluble salts and elemental mercury, followed by analysis. Standards of BSIA as a sodium salt were freshly prepared and for BSOA by preparation of an approximately 0.1 *M* solution in 50% methanol, standardisation of this with 0.1 *M* sodium hydroxide using phenolphthalein indicator and dilution to an appropriate concentration.

Degradation of diphenylmercury

To an ampoule containing 5 ml of 0.1% (w/v) sodium metabisulphite were added metallic mercury (approximately 50 mg) and 50 µl of $1 \cdot 10^{-2}$ *M* DPM in acetonitrile (equivalent to $1 \cdot 10^{-4}$ *M* DPM), and the ampoules were autoclaved at 121°C for 1.5 h. The supernatants were submitted to analysis for PM nitrate, DPM, BSOA and BSIA.

RESULTS AND DISCUSSION

Initial studies demonstrated that DPM was formed as an intermediate in the degradative process, and to assess its importance a kinetic study was performed at 100°C, the concentrations of PM nitrate, DPM and Hg^{II} being monitored. The use of DEADTC as complexing agent allows separate quantitation of the PM nitrate (4.5 min) and Hg^{II} complex (6.1 min) with DPM co-eluting with a minor peak at 3.6 min which is due to the corresponding thiuram disulphide arising from the slow atmospheric oxidation of the DEADTC (Fig. 2)[11]. The method affords a linear response over the range 0–$2.5 \cdot 10^{-4}$ *M* for both PM nitrate ($n = 6$, $r = 0.9998$; coefficient of variation, C.V. = $\pm 0.93\%$ at $0.5 \cdot 10^{-4}$ *M*, $n = 6$) and Hg^{II} ($n = 6$, $r = 0.9996$; C.V. = $\pm 1.80\%$ at $0.5 \cdot 10^{-4}$ *M*, $n = 6$). The DPM thus had to be quantitated using MDTC reagent, the more polar complexes of both the PM ion and Hg^{II} eluting prior to the uncomplexed DPM, and therefore not interfering with the assay (Fig. 3). The method affords a linear response over the range 0–$1 \cdot 10^{-4}$ *M* for DPM ($n = 5$, $r = 0.9999$; C.V. = $\pm 2.9\%$ at $1 \cdot 10^{-5}$ *M*, $n = 6$).

Application of these analytical methods to the PM nitrate–bisulphite solution

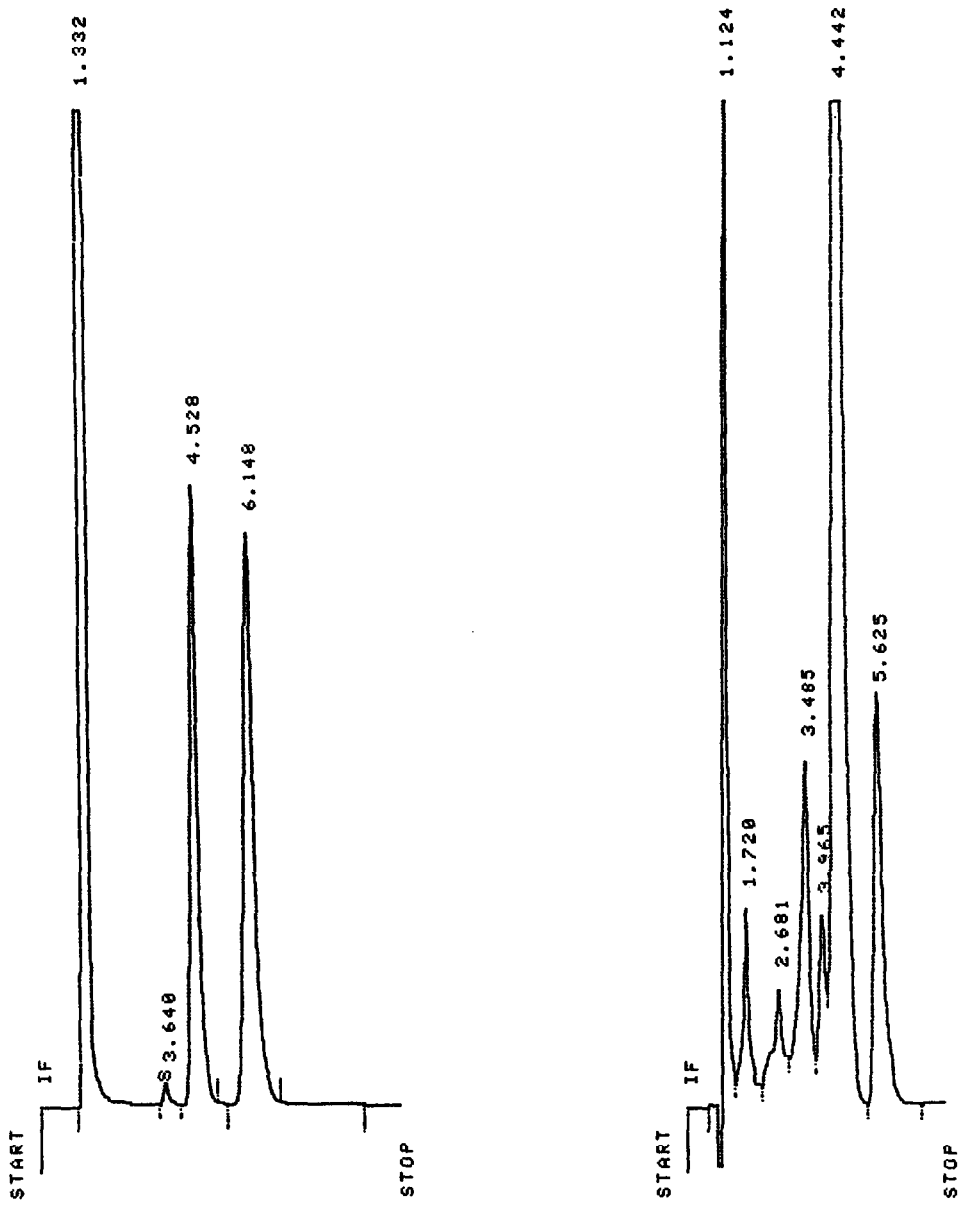

Fig. 2. Chromatogram of a solution of PM nitrate and Hg^{II} acetate, each $1 \cdot 10^{-4}$ *M* (0.064 a.u.f.s., monitoring wavelength 258 nm). Peaks: 1.33 min, excess DEADTC reagent; 3.64 min, DEADTC oxidation product; 4.53 min, PM–DEADTC complex; 6.15 min, Hg–DEADTC complex. Numbers at peaks are retention times in min.

Fig. 3. Chromatogram of a degraded sample of PM nitrate after heating at 100°C for 40 min (0.016 a.u.f.s., monitoring wavelength 225 nm). Peaks: 1.12 min, excess MDTC reagent; 3.48 min, MDTC oxidation product; 3.96 min, Hg–MDTC complex; 4.44 min, PM–MDTC complex; 5.62 min, DPM. The peaks at 1.72 and 2.68 min are unidentified degradation products of the MDTC reagent.

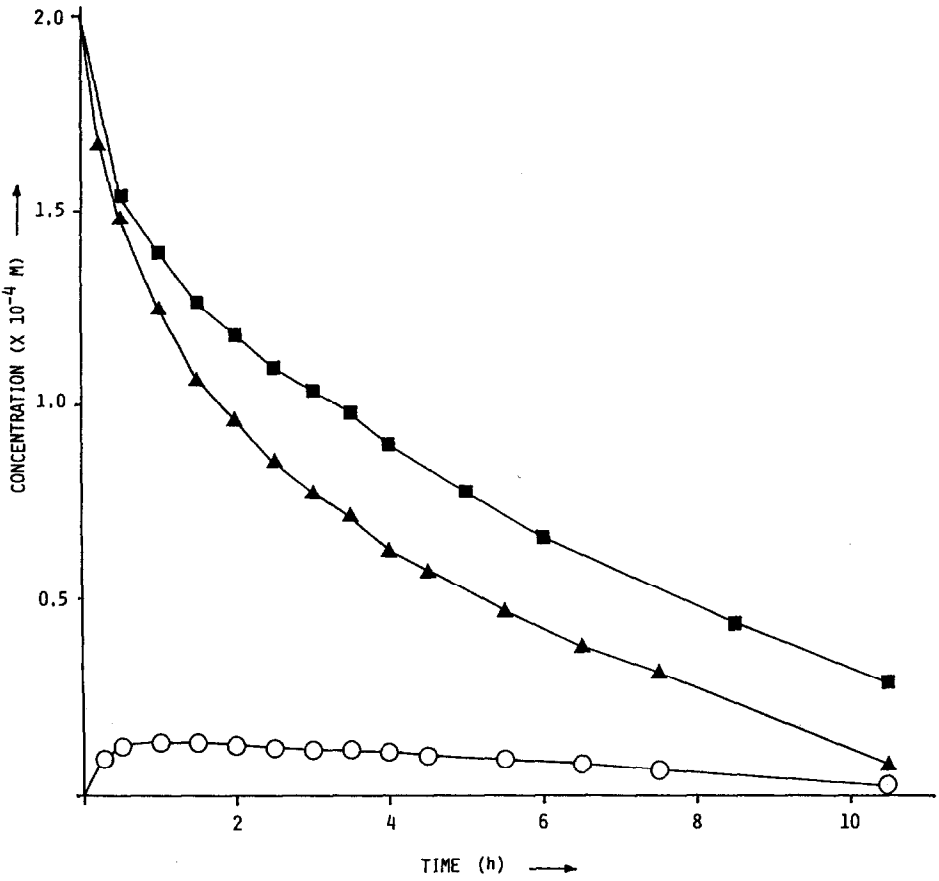

Fig. 4. Concentration of PM nitrate remaining following heating at 100°C with sodium metabisulphite. ■ = Amethocaine eye drops APF containing $2 \cdot 10^{-4}$ *M* PM nitrate; ▲ = $2 \cdot 10^{-4}$ *M* PM nitrate solution. (○) Concentration of DPM arising from heating the $2 \cdot 10^{-4}$ *M* PM nitrate solution.

showed that at 98–100°C there was a rapid loss of PM nitrate accompanied by the formation of DPM (Fig. 4). Similar results were obtained for the amethocaine eye drops[9]. The DPM was not quantitated for these drops as a minor impurity in the amethocaine co-eluted with the DPM, which could, however, be identified as being present. In all studies the level of soluble mercury salts remained below 2% of total mercury and was not quantitated. In this study no attempt was made to quantitate the insoluble elemental mercury formed during the degradation. PM nitrate solution, in the absence of sodium metabisulphite, when submitted to the same conditions afforded no significant loss ($1.97 \cdot 10^{-4}$ *M*, corresponding to a loss of 1.5% after 10 h).

These observations confirm that PM salts in the presence of metabisulphite undergoes a chemical reaction and that the observations of previous workers arise from a true degradation and not from complex formation affecting the AAS method. The identity of the DPM was confirmed by comparison of its chromatographic retention characteristics with those of an authentic sample in this system, in the solvent

TABLE I

PERCENTAGES OF INORGANIC MERCURY FORMED FOLLOWING AUTOCLAVING OF PHENYLMERCURIC NITRATE SOLUTIONS

Results of two or three experiments followed by the mean percentage in parentheses.

Sample	*Percentage of inorganic mercury*	
	Soluble	*Elemental and insoluble salts*
$2 \cdot 10^{-4}$ *M* PM nitrate[a]	1.5, 1.4 (1.4)	39.3, 42.7 (41.0)
Amethocaine eye drops APF containing $2 \cdot 10^{-4}$ *M* PM nitrate	7.3, 3.4 (5.4)	14.0, 17.0 (15.5)
$2.5 \cdot 10^{-4}$ *M* PM nitrate[b]	1.5, 1.2, 1.0 (1.2)	90.0, 89.6, 84.4 (88.0)

[a] In these studies supernatants were removed by aspiration prior to digestion with nitric acid of the invisible residue.

[b] In this study the total contents of the ampoule were subjected to nitric acid digestion.

system employed for the PM–DEADTC analysis and also using methanol–water (75:25) as a mobile phase. A UV spectrum was obtained of the DPM using a diode-array spectrophotometer in series with the HPLC system and this was superimposable on that of an authentic spectrum of DPM obtained in a similar manner.

Investigation of the species of mercury present following degradation has been investigated by prolonged autoclaving to ensure complete destruction of the PM nitrate and quantitation of the water-soluble and -insoluble fractions. The results are displayed in Table I. Attempts to remove the supernatants following centrifugation and prior to digestion to dissolve elemental mercury resulted in substantial losses whereas digestion of the total contents of an ampoule demonstrated that 88% of total mercury could be accounted for as the Hg^{II} ion.

Studies have also been undertaken into the nature of the degradation products arising from the phenyl portion of the PM nitrate. Preliminary studies indicated that the products of degradation of the PM ion are BSOA and BSIA, and this was confirmed by comparison of retention times with authentic samples in two HPLC systems (33% methanol containing 0.1% tetrabutylammonium hydrogensulphate; 25% acetonitrile containing 0.1% tetrabutylammonium hydrogensulphate) and comparison of UV spectra obtained using a diode-array spectrophotometer in series with the HPLC system with authentic samples.

The BSOA and BSIA were quantitated by HPLC. The system afforded three peaks due to nitrate (3.2 min), BSOA (5.9 min) ($r = 0.9999$ over the concentration range $0–4.8 \cdot 10^{-4}$ *M*, $n = 5$; C.V. at $2.4 \cdot 10^{-4}$ *M* $= \pm 1.3\%$, $n = 6$) and BSIA (7.4 min) ($r = 0.9999$ over the concentration range $0–5 \cdot 10^{-4}$ *M*, $n = 5$; C.V. at $2.5 \cdot 10^{-4}$ *M* $= \pm 0.9\%$, $n = 6$) with baseline resolution (Fig. 5). Samples of PM nitrate following prolonged autoclaving with sodium metabisulphite were submitted to analysis for the acids under different conditions (Table II). Only about 75% of the PM nitrate could be accounted for by formation of the acids, and the exact proportion of the two acids varied with the conditions. In sealed ampoules the proportion of products was consistent and when exposed to the atmosphere oxidation occurred of

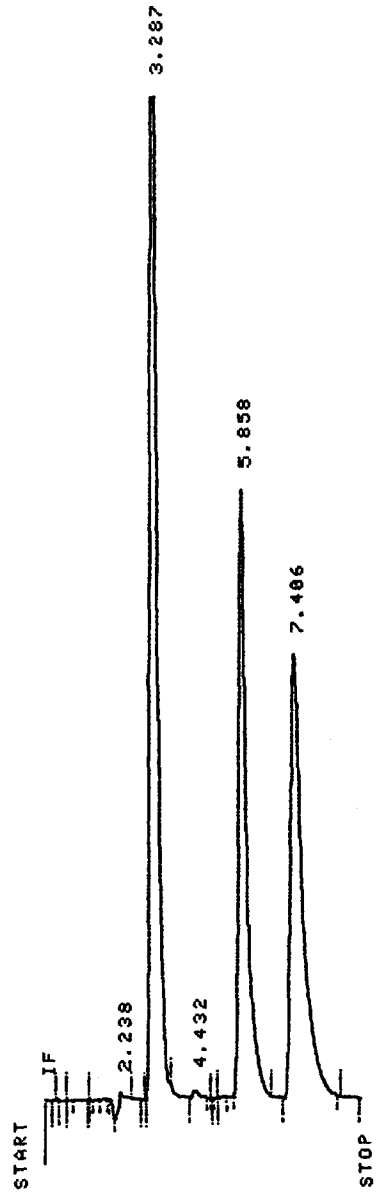

Fig. 5. Chromatogram of a sample of PM nitrate ($2.5 \cdot 10^{-4}$ *M*) autoclaved at 121°C for 2 h (0.064 a.u.f.s., monitoring wavelength 220 nm). Peaks: 3.29 min, nitrate; 5.86 min, BSOA; 7.41 min, BSIA.

the BSIA to BSOA. Sulphinic acids are known to undergo rapid atmospheric oxidation to the corresponding sulphonic acids[13] and this may occur following or together with atmospheric oxidation of the residual sodium metabisulphite. This would account for the increased proportion of BSOA encountered in the loosely stoppered flasks to which oxygen has access at the end of the autoclave cycle. Together

with the BSOA and BSIA approximately 1% of the PM nitrate is degraded to phenol which is just detectable under the conditions used in this study (signal-to-noise ratio = 3).

The most plausible explanation for the transient formation of DPM together with elemental mercury, BSOA and BSIA is that under the extreme conditions of heat sterilisation the bisulphite ion reduces the PM ion to the mercurous form which in turn undergoes disproportionation to DPM and elemental mercury (Fig. 1). It has been previously demonstrated that organomercurous radicals are in equilibrium with dialkyl- and diarylmercury compounds in the presence of metallic mercury via a process of homolytic fission which takes place at the metal surface[14–17]. Homolytic fission of the phenyl–mercury bond of the intermediate organomercurous radical would afford elemental mercury and a reactive phenyl radical, this demercuration reaction probably taking place at the metal surface as for the disproportionation reaction. The phenyl radical could then react with other species in the system to give the range of poducts noted. Bisulphite is known to exist in solution as a complex mixture of species the exact composition of which is influenced by pH, temperature and concentration[18], and reaction with these affords the BSOA and BSIA. When DPM is autoclaved in the presence of sodium metabisulphite and metallic mercury analysis of the product mixture showed the absence of DPM and PM nitrate and the formation of BSOA and BSIA in proportions roughly equivalent to those found in the degradation of PM nitrate solutions (Table II), an observation which supports the proposed mechanism.

The fact that elemental mercury is found in these degradative processes may account for the differing results obtained by Richards *et al.*[2] and other workers[1,5,6] when measuring PM salts by AAS. It is reasonable to suppose that PM nitrate degrades in amethocaine eye drops[9] by a similar mechanism, and upon prolonged autoclaving in the presence of amethocaine hydrochloride (Table II) affords BSOA and BSIA, the change in proportion of acids being probably ascribable to a pH effect on the species in the system arising from the bisulphite[17]. It is obvious from these studies that the amethocaine eye drops[9] are unsatisfactory and that sodium

TABLE II

PERCENTAGES OF PHENYLMERCURY AND DIPHENYLMERCURY CONVERTED TO BENZENESULPHONIC AND BENZENESULPHINIC ACIDS UNDER DIFFERENT CONDITIONS OF AUTOCLAVING

Values are means of three experiments.

Sample	*Sulphonic acid*	*Sulphinic acid*	*Total acids*
PM nitrate in sealed ampoules	39.7	33.0	72.7
PM nitrate in unsealed ampoules[a]	47.0	25.3	72.3
PM nitrate in loosely stoppered flasks	54.3	19.3	73.6
PM nitrate in sealed ampoules plus amethocaine hydrochloride (1%, w/v)	3.7	64.7	68.4
DPM in sealed ampoules[b]	54.6	27.7	82.3

[a] Preceding ampoules exposed to the atmosphere for 2 days and reassayed.
[b] Calculated to the basis of 1 mol of DPM affording 2 mol of acids.

metabisulphite should never be used as an antioxidant together with PM nitrate as an antimicrobial preservative in ophthalmic products.

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