International Journal of Pharmaceutics, 48 (1988) 167–172
Elsevier

IJP 01626

Polarographic analysis of taurolidine, a non-antibiotic antimicrobial agent

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(Received 1 March 1988)
(Accepted 1 May 1988)

Key words: Taurolidine; Taurultam; Differential pulse polarography

Summary

The behaviour in aqueous solution of the non-antibiotic antimicrobial agent, taurolidine, which has marked anti-adherence properties, has been investigated by differential pulse polarography (DPP). Taurolidine gave well-defined differential pulse polarograms at pH 4.2 with a peak potential of −0.83 V vs Ag/AgCl. This behaviour was identical to that of aqueous taurultam solutions. Comparison of peak current ratios, cyclic voltammograms and peak potential/pH plots confirmed that the taurolidine signal was due to the reduction of taurultam and its hydroxymethyl derivative. A mechanism for the cathodic reduction of taurultam was proposed involving a 2-electron transfer. The signal at −0.83 V was of analytical utility, but was lost at alkaline pH. A second peak appeared in the polarogram for taurolidine solutions at alkaline pH and was identified as formaldehyde. This was quantified by a rapid DPP method. Only trace amounts of formaldehyde were found in commercial taurolidine (Taurolin) solutions and these were of no clinical significance.

Introduction

Taurolidine (bis(1,1-dioxoperhydro-1,2,4, thiazinyl-4-)-methane is a broad spectrum, non-antibiotic agent derived from the aminosulphonic acid, taurine. Taurolidine is available in Western Europe as a 2% solution containing 5% polyvinylpyrrolidone (Taurolin). It is intended for local use, particularly intraperitoneally and in the bladder. Intraperitoneal administration of taurolidine has been shown to significantly reduce morbidity in peritonitis (Browne et al., 1978; Browne, 1981). Recently, Gorman et al. (1987) and Woolfson et al. (1987) have demonstrated that taurolidine has significant anti-adherence activity against several microbial species. The concept of an agent which can prevent or control infection by interfering with the adhesion process has prompted increased interest in those antimicrobial agents, such as taurolidine, which possess this property.

Studies on taurolidine, both in vitro and in vivo, have been hindered by the lack of a suitable, routine analytical method. The behaviour of taurolidine in aqueous solution has been studied by PMR spectroscopy (Myers et al., 1980; Knight et al., 1983). A complex equilibrium was proposed in which taurolidine was largely degraded to a mixture of taurultam (1,1-dioxoperhydro-1,2,4-thiadiazine) and hydroxymethyltaurultam, together with trace amounts of formaldehyde (Fig.

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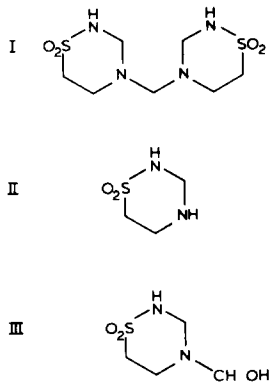

Fig. 1. Chemical structures of taurolidine (I), taurultam (II) and hydroxymethyltaurultam (III).

1). These equilibria were probably further influenced by the presence of polyvinylpyrrolidone in commercial solutions. Knight et al. (1981) had earlier proposed a high-performance liquid chromatographic assay for taurolidine involving pre-column derivatisation with dansyl chloride and subsequent UV detection. Taurultam and taurineamide, a metabolite, were detected in patient plasma samples following administration of the commercial solution (Taurolin). However, the derivatisation reaction also converts taurolidine to taurultam, so taurolidine could not be detected by this method (Erb et al., 1983).

The electroanalysis of taurolidine and related compounds has not so far been investigated. The present study therefore reports a differential pulse polarographic study of taurolidine solutions, together with a rapid, direct polarographic determination of trace formaldehyde in taurolidine solutions.

Materials and Methods

Chemicals

Taurolidine and taurultam were kindly supplied by Geistlich & Sons (Chester, U.K.). All other reagents were of Analar or equivalent quality.

Preparation of standard formaldehyde solutions

Standard formaldehyde solutions were prepared by appropriate dilution of Analar formaldehyde solution (B.D.H. Chemicals, Poole, U.K.) which was standardised by sodium sulphite assay (Walker, 1964).

Buffer solutions

Universal buffer solutions for polarography (taurolidine and taurultam) were Prideaux buffers prepared as previously described (Baxter et al., 1984). For the polarographic determination of formaldehyde the supporting electrolyte was 0.1 M lithium hydroxide.

Instrumentation

Polarographic determinations were made with a PAR 174A polarograph (E.G.&G Princeton Applied Research, Princeton, U.S.A.) and a conventional 3-electrode system comprising a dropping mercury electrode (DME) equipped with a drop timer, Ag/AgCl reference and platinum auxiliary electrodes. The polarographic cell was of 20 ml capacity and all solutions were degassed with oxygen-free nitrogen for 5 min. Polarograms were recorded on an EW-11 x–y plotter (Rikadenki-Mitsui, U.K.).

Cyclic voltammetry was performed with a Metrohm E506 polarograph and E 612 function generator (Metrohm, Herisau, Switzerland). A Metrohm E 663 multi-mode electrode in the H.M.D.E. mode was used and output was via a Gould 2001 digital storage oscilloscope and x–y plotter.

Determinations of pH were made with a Model 701A meter and 9015 gel-filled combination glass electrode (Orion Research, U.S.A.).

Polarographic conditions

Initial potential: -0.5 V vs Ag/AgCl (for formaldehyde -1.0 V vs Ag/AgCl); drop time: 1 s; current sensitivity: 2 or 5 μA as required; potential scan rate: 5 mV $\cdot$ s^{-1}; modulation amplitude: 50 mV. For cyclic voltammetry a scan rate of 0.5 V $\cdot$ s^{-1} was used between -1.5 and $+1.5$ V.

Results and Discussion

Differential pulse polarograms were obtained through the pH range 3–9. Both taurolidine and

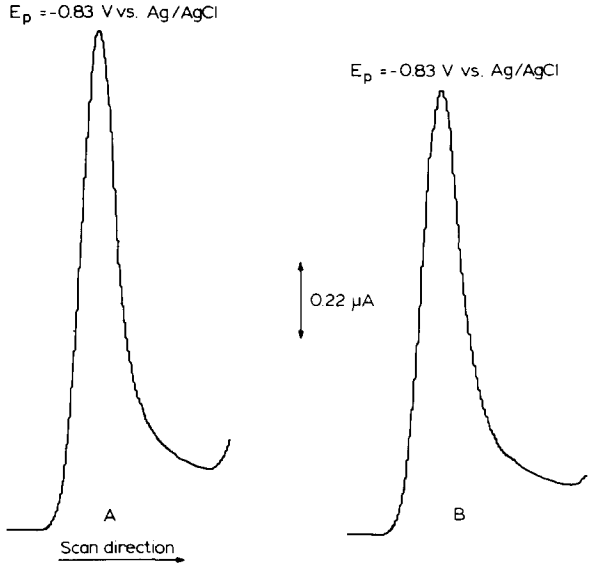

Fig. 2. Differential pulse polarograms of (A) taurolidine (0.1% m/V, 3.5×10^{-3} M) and (B) taurultam (0.1% m/V, 7.35×10^{-3} M), both in pH 4.2 Prideaux buffer, initial potential −0.5 V vs Ag/AgCl.

taurultam (0.1% mV) gave well-defined signals (Fig. 2A and B) at pH 4.2 with identical peak potentials (E_p) of −0.83 vs Ag/AgCl. These peaks disappeared in supporting electrolytes of pH > 7.5. However, for taurolidine alone a second well-defined differential pulse signal was obtained at pH 8.7 with an E_p of −1.40 vs Ag/AgCl (Fig. 3). Plots of E_p vs pH (Figs. 4 and 5) were linear

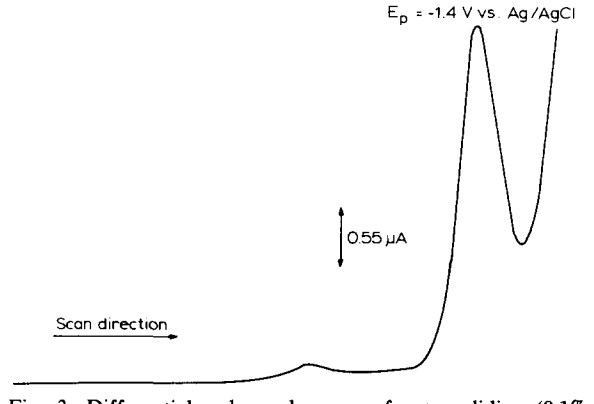

Fig. 3. Differential pulse polarogram for taurolidine (0.1% m/V) in pH 8.7 Prideaux buffer, initial potential −0.5 V vs Ag/AgCl.

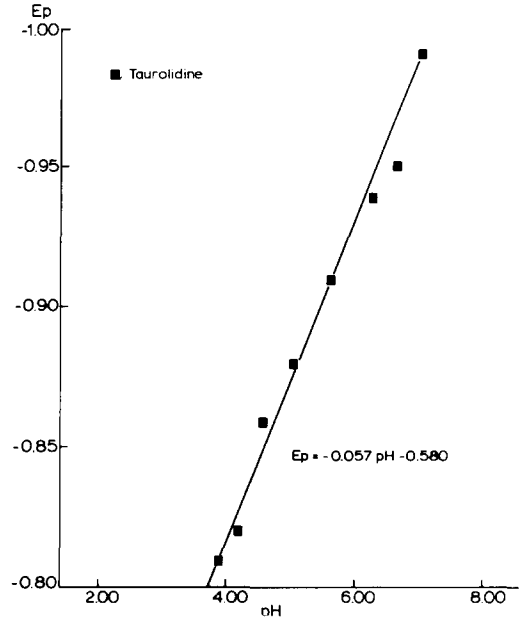

Fig. 4. Variation of peak potential (E_p) with pH for taurolidine (0.1% m/V).

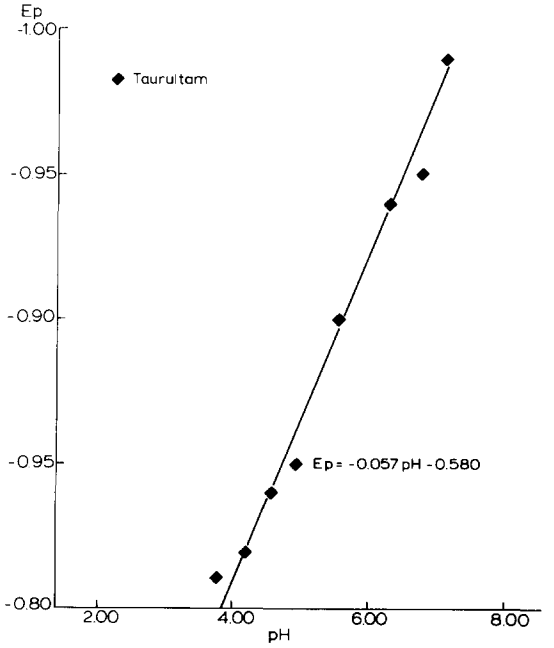

Fig. 5. Variation of peak potential (E_p) with pH for taurultam (0.1% m/V).

($r > 0.99$) for both taurolidine and taurultam. Regression analysis gave identical slopes of 57 mV for both compounds.

Peak current vs concentration plots were linear ($r > 0.99$) for taurolidine and taurultam, both in the concentration range 0.05–0.1% m/V. Cyclic voltammograms were rather poorly defined for both compounds (Fig. 6) and showed only one similar peak in each case on the cathodic section of the scan, indicating that the reduction of both taurolidine and taurultam proceeds irreversibly at the D.M.E. The slope of 57 mV for the E_p vs pH plots for both compounds corresponds to a 1 : 1 proton : electron ratio for the reduction process. These observations, together with the identical E_p values and cyclic voltammograms, confirm that taurolidine in aqueous solution exists almost entirely in the form of taurultam, and that the differential pulse polarograms for these compounds are due to the reduction at the D.M.E. of taurultam. From Fig. 2A and 2B a peak current ratio taurolidine : taurultam of 1.125 : 1 is obtained for concentrations of 3.5×10^{-3} M taurolidine and 7.35×10^{-3} M taurultam. If one mole of taurolidine yielded two electroactive moieties, a theoretical peak height ratio of 7.0 : 7.35, i.e. 0.952 would be expected, close to the actual value obtained. The difference can be ascribed to the complex nature of the equilibria involved (Knight et al., 1983).

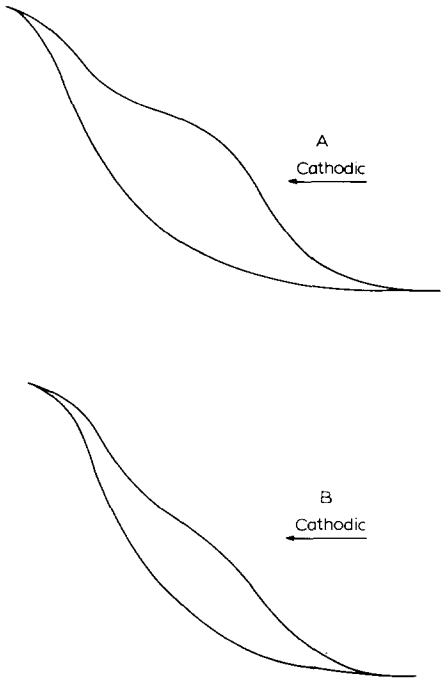

Fig. 6. Cyclic voltammograms for taurolidine (A) and taurultam (B) between −1.5 V and +1.5 V at 0.5 V·s^{-1}.

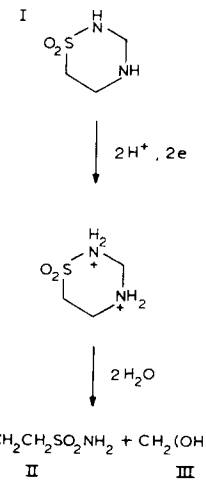

Fig. 7. Proposed electrochemical reaction scheme for the polarographic reduction of taurultam (I) to taurineamide (II) and methylene glycol (III).

On the basis of the results obtained, a mechanism can be postulated for the polarographic reduction of taurolidine and taurultam. The dimer degrades on aqueous dissolution to taurultam and hydroxymethyltaurultam, these two compounds being indistinguishable polarographically. Since the reduction step only occurs at acidic pH, it is reasonable to conclude that protonation of the ring nitrogen atoms is a prerequisite for the cathodic reduction to occur. The proposed reduction reaction (Fig. 7) then proceeds with ring opening via the transfer of two protons and two electrons, consistent with the 1 : 1 proton : electron ratio indicated by the E_p vs pH plots. The products of the electroreduction are therefore taurineamide (a precursor of taurultam) and methylene glycol (hydrated formaldehyde), the starting materials for the synthesis of taurultam.

Aqueous solutions of taurultam stored for prolonged periods at room temperature, and subsequently diluted with pH 4.2 buffer and polarographed, showed no change in peak current, even

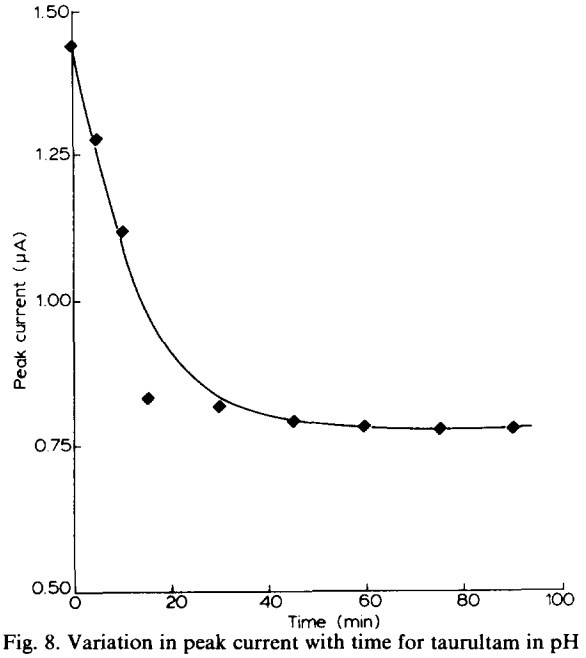

Fig. 8. Variation in peak current with time for taurultam in pH 3 Prideaux buffer.

after autoclaving. However, taurultam stored at a pH of 3.0 showed a substantial reduction in peak current over about a 45-min period leading to the establishment of an equilibrium position (Fig. 8) at approximately 50% of the original taurultam concentration. A similar result was obtained when taurultam solutions were prepared in dilute mineral acid. This situation can be explained on the basis of protonation of the ring nitrogens and subsequent ring opening to establish an equilibrium between taurultam and the reaction products, taurineamide and methylene glycol.

The presence of a second peak in alkaline buffer solutions of taurolidine, but not taurultam, is of some interest. The position of this signal at -1.40 V vs Ag/AgCl corresponds to the polarographic reduction of formaldehyde previously observed (Woolfson et al., 1985). Formaldehyde is only reduced polarographically following the dehydration of methylene glycol at alkaline pH, yielding a kinetic current (Woolfson et al., 1985). Standard formaldehyde solutions in 0.1 M lithium hydroxide as supporting electrolyte yielded identical signals to the well-defined differential pulse polarogram obtained with taurolidine in this electrolyte. Formaldehyde calibrations were linear over the range $1 \times 10^{-5}\%$ to $6 \times 10^{-5}\%$ ($r > 0.99$). Freshly prepared taurolidine solutions and Taurolin (commercial solution containing 2% m/V taurolidine in 5% polyvinylpyrrolidone) were analysed for their formaldehyde concentrations (direct dilution with 0.1 M LiOH). Five replicate analyses for taurolidine gave a mean formaldehyde concentration for a 2% solution of $7.8 \times 10^{-4}\%$ ($s = 1.1 \times 10^{-5}$) compared with $1.95 \times 10^{-3}\%$ ($s = 8.3 \times 10^{-5}$) in the commercial 2% taurolidine solution (Taurolin). These values compare to $3.7 \times 10^{-3}\%$ formaldehyde in taurolidine (2%) solutions reported by Knight et al. (1983) using capillary gas chromatography with head space analysis. Values for commercial Taurolin were not reported by these authors due to interference in the method by polyvinylpyrrolidone. Thus, the present study shows that the commercial solution contained 2.5 times more formaldehyde than its freshly prepared equivalent but that the amount of formaldehyde was still clinically insignificant, in agreement with the conclusions of Knight et al. (1983). By contrast, Nash's method (Nash, 1953) appears to release the total available formaldehyde from taurolidine, i.e. 3 formaldehyde molecules per taurolidine molecule (Myers et al., 1980). The origin of formaldehyde in these solutions is probably via breakdown in aqueous solution of hydoxymethyltaurulatam to taurultam with consequent liberation of hydrated formaldehyde. Thus, formaldehyde is absent from aqueous taurultam solutions. The small amounts of formaldehyde present in taurolidine solutions suggests that the further equilibrium between taurultam and hydroxymethyltaurultam suggested by Myers et al. (1980) largely favours the hydroxymethyl derivative. Nevertheless, this compound has never actually been isolated from taurolidine solutions. However, the previously unreported observation that taurultam is partially degraded in acidic solution with consequent liberation of formaldehyde lends some support to the original suggestion by Browne et al. (1976) that bacterial cell enzyme activity may be responsible for liberation of formaldehyde in situ from taurultam, and this may contribute to the antimicrobial actions of taurolidine solutions.

The present study presents a new analytical method for taurolidine (as taurultam), confirms that taurolidine in aqueous solution largely exists as taurultam and its hydroxymethyl derivative and provides a direct, rapid analysis for the trace formaldehyde concentration of commercial Taurolin solutions. The method does not detect taurultam in the presence of polyvinylpyrrolidone due to a direct passivating effect on the D.M.E. However, at the high dilutions employed, polyvinylpyrrolidone does not interfere with the formaldehyde assay. This polarographic study may provide the basis for the direct electrochemical detection of these compounds by high-performance liquid chromatography.

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