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Short Communication

Adsorptive stripping voltammetry for thiomersal assay

P. GRATTERI,*† S. FURLANETTO,† S. PINZAUTI,† E. LA PORTA,† P. MURA† and G. SANTONI‡

†*Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via G. Capponi 9, 50121 Firenze, Italy*
‡*Stabilimento Chimico Farmaceutico Militare, Via R. Giuliani 201, 50141 Firenze, Italy*

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Introduction

Thiomersal, the sodium salt of (2-carboxyphenylthio)ethylmercury, is a well known bacteriostatic and fungistatic agent that is commonly used as a preservative in biological products. Thiomersal is also widely used in the formulation of hard and soft contact lens solutions [1].

Studies of the interaction of thiomersal with solid surfaces have been restricted to plastic containers [2], nitrocellulose membrane filters [3] and polymers constituting the contact lens material [4]. The uptake of thiomersal by polyhydroxyethylmethacrylate was found to be pH-dependent, with no uptake occurring unless some thiomersal is present in the unionized form, i.e. below pH 5.0 [4]. Accordingly, severe loss of thiomersal from neutral contact lens solutions packaged in polyolefinic containers was attributed to sorption of the antibacterial agent and its degradation products in the polymer network rather than to adsorption onto the plastic surface, since no interaction is to be expected between a carboxylate anion and a polymeric hydrocarbon [2].

From an electroanalytical point of view the interaction of an analyte at the mercury surface takes on a particular interest, controlled adsorptive accumulation onto the surface of the hanging mercury drop electrode (HMDE) being an effective pre-concentration step prior to the highly-sensitive and selective voltammetric measurement in the differential-pulse stripping mode [5, 6].

The polarographic behaviour of thiomersal has previously been discussed [7–9] and the reduction process is suitable for its assay in concentrations up to 2.5 μM in the differential-pulse mode [9] without interference from photodegradation products [8]. The present paper describes an adsorptive stripping voltammetric (AdSV) procedure developed for the assay of thiomersal with a lower determination limit of 5×10^{-8} M. The method was applied to the determination of thiomersal in contact lens solutions.

Experimental

Materials

All solutions were made using double-distilled water passed through a Millipore Milli-Q system. An aqueous stock solution (1.2 mg ml^{-1}) of thiomersal (standard sample used as received from Eli Lilly, Italy) was prepared daily and stored in the dark. As supporting electrolyte a Britton–Welford buffer was used: sodium hydroxide–monobasic potassium phosphate (0.1 M) (40:100, v/v) (pH* 7.0). A simulated contact lens solution was made up by dissolving 1 mg of thiomersal, 0.1 g of disodium edetate, 0.6 g of sodium chloride, 0.2 g of boric acid and 0.175 g of borax in 100 ml of water. Five brands of commercial contact lens solutions were

* Author to whom correspondence should be addressed.

selected as being representative of products currently marketed in Italy, and were purchased locally.

Apparatus

Adsorptive and voltammetric experiments were performed on a Metrohm 646 VA Processor coupled with a VA-647 stand which incorporates a multi-mode mercury electrode assembly (DME or HMDE), as working electrode, with a high capacity for reproducible production of the hanging mercury drops. The three-electrode system was completed with an Ag/AgCl reference electrode and a platinum rod as the auxiliary electrode. A built-in vertical Teflon-coated rod stirrer was used for stirring during the accumulation step. The optimum instrumental parameters were as follows: accumulation potential, −350 mV; accumulation time with stirring, 30 s; drop size, 0.6 mm^2; stirrer speed, 1920 rev. min^{-1}; potential scan rate, 10 mV s^{-1}; pulse amplitude, 50 mV; rest time after accumulation, 15 s. Because of the possibility of thiomersal photodegradation, the measuring cell (neutral glass) was protected from light during the analysis. Cyclic voltammograms at a static mercury drop electrode were performed with an Amel 473 polarographic analyser connected to a digital Amel *X–Y* recorder model 863 and using an Ag/AgCl as reference electrode and a platinum wire as auxiliary electrode. Highly purified nitrogen was passed through the solution to remove dissolved oxygen.

Procedure

The determination of thiomersal was performed by means of a calibration graph prepared as follows: an aliquot of thiomersal stock solution (0.5 ml) was transferred into a 100-ml calibrated flask and diluted to volume with water. This standard solution (6 μg ml^{-1}) was used for AdSV experiments. An accumulation potential of −350 mV was applied for 30 s to the working electrode in a known volume (15 ml) of supporting electrolyte (Britton–Welford buffer, previously degassed with a stream of purified nitrogen for 10 min, and for only 30 s between succesive adsorptive cycles) while stirring the solution continuously. The stirring was then stopped and, after a 15 s rest period, the stripping voltammogram was recorded, with application of a differential-pulse ramp in the negative direction (from −0.35 to −0.90 V). First, a background voltammogram was recorded; then a further nine voltammograms were recorded for in-cell additions of the standard thiomersal solution, in accordance with the scheme: 50 μl for the first addition, 25 μl for the second and third and 50 μl for the remaining additions. For each analyte concentration the adsorptive stripping cycle was repeated at least twice using a new mercury drop. The entire procedure was automated with control by means of the programming capacity of the apparatus. All experiments were carried out at room temperature. Commercial lens solutions were assayed, without the need for previous extraction and under AdSV conditions described above, by pipetting 120 μl of each preparation into the cell containing 15 ml of supporting electrolyte.

Results and Discussion

The adsorptive accumulation effect of thiomersal has been tested by recording voltammograms of a sample solution with and without the imposition of an increasing accumulation time under stirring conditions. Thiomersal adsorption onto the electrode surface is demonstrated by the enhancement of the peak current, which is due to the accumulation step (Fig. 1). As expected, deviations from linearity of the current–time graph were observed as complete surface coverage was approached

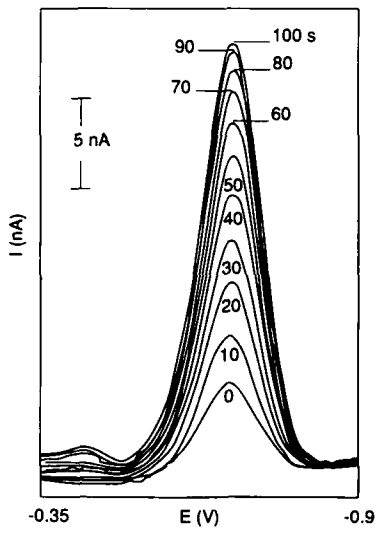

Figure 1
Differential pulse adsorptive stripping voltammograms with variable accumulation times (from 0 to 100 s at 1920 rpm) for 3.5×10^{-7} M thiomersal. Accumulation potential, −0.35 V versus Ag/AgCl; scan rate, 10 mV s^{-1}; pulse amplitude, 50 mV; supporting electrolyte, Britton–Welford buffer (pH 7).

(from data of Fig. 1, at pre-concentration periods of over 50 s at the 3.5×10^{-7} M concentration level). The same process was observed with cyclic voltammetric studies with and without accumulation. Cyclic voltammograms also showed the irreversibility of the electrolytic process. After a short pre-concentration time, repetitive cyclic voltammograms gave a higher first scan peak current than those obtained with subsequent scans on the same drop and thiomersal solution. This means that there was a rapid desorption of the analyte from the electrode surface. The cathodic peak current increased linearly as the potential scan rate was increased from 10 to 200 mV s^{-1}. The corresponding plot of log peak current versus log scan rate had a slope of 0.70, an acceptable value considering that slopes of 1 and 0.50 are expected for ideal reactions of surface and solution species, respectively [10], and that in most cases the voltammetric behaviour of surface-confined species is not ideal [11]. Thus, the spontaneous adsorption of the electroactive thiomersal onto the electrode surface allowed its effective stripping voltammetric trace measurement.

The adsorption process did not exhibit a strong dependence upon the applied potential; there was a strong thiomersal adsorption in the range of potential considered (from −0.30 to −0.45 V). Thus, a −0.35 V accumulation potential was chosen, the same as the starting potential and well above the stripping peak potential of −0.68 V. The effect of pH (in the range 2–9) was investigated by absorptive stripping voltammetry of thiomersal in different buffers of different ionic strength. The highest peak current was found at pH 7. At this pH, Britton–Welford buffer was selected as the best supporting electrolyte. Mass transport during the pre-concentration step affected the amount of thiomersal adsorbed and the resulting stripping response. Stirring proved to give substantial enhancement (2–3 fold) of the peak height.

Thiomersal quantitation was possible by the linear dependence of the peak height on the concentration of the antibacterial present in the solution. The calibration line (95% confidence limits for intercept and slope) gave the equation: $y = 3.79\ (\pm 0.0473)x - 0.185\ (\pm 0.127)$. The detection limit was calculated as the analyte concentration giving a signal equal to the blank signal y_B (the intercept) plus three standard deviations of y-residuals $s_{y/x}$, and was equal to 5.48×10^{-9} M. The precision of the method, expressed as the value of the relative standard deviation calculated from the triplicate measurements carried out for eight 3×10^{-7} M solutions of thiomersal, was 3.14%.

Since the addition of 120 μl of each commercial preparation examined gave an approximate in-cell concentration of 2×10^{-7} M, quantification of thiomersal in contact lens solutions was performed by means of a calibration graph using a more restricted concentration range ($1–3 \times 10^{-7}$ M) than that described previously for the linearity range. Table 1 shows the slope, intercept and correlation coefficient of the calibration curve used for assaying contact lens solutions. In Table 1 the concentration values of the calibration graph are expressed in mg 100 ml^{-1}, i.e. as on the labels of pharmaceutical preparations. Table 1 also gives the results of the analyses of commercial contact lens solutions containing common buffering, chelating, isotonicity and

Table 1
Data of calibration curve and results for AdSV determination of thiomersal in commercial contact lens solutions

Conc. range (mg 100 ml^{-1})	Sensitivity (nA 100 ml mg^{-1})	Intercept (nA)	Correlation coefficient (n = 5)	Detection limit (mg 100 ml^{-1})	Actual in-cell conc. range (M)
0.505–1.49	7.13 ± 0.13	0.63 ± 0.138	0.9999	0.018	$0.99–2.9 \times 10^{-7}$

Sample	Thiomersal claimed (mg 100 ml^{-1})	Thiomersal found (mg 100 ml^{-1}) ($\pm ts_{x_0}$, n = 4)	Found % (RSD, n = 4)	Recovery % of thiomersal by an alternative method [9] (RSD, n = 4)
A	1	0.98 (0.03)	99.3 (2.8)	99.7 (2.9)
B	1	1.08 (0.03)	108.0 (3.1)	109.2 (2.3)
C	1.15	1.01 (0.02)	101.5 (3.5)	102.6 (2.6)
D	1	1.21 (0.03)	121.0 (3.4)	123.0 (3.0)
E	1	0.80 (0.02)	80.0 (2.5)	79.3 (2.9)
Simulated solution	1	0.99 (0.02)	99.1 (3.0)	99.4 (2.1)

viscosity-increasing agents, as compared with those of a previously-described differential pulse polarographic method [8]. The use of the regression line reported may involve an error in the calculation of a concentration value (x_0) from a measured peak current value because both the slope and the intercept are subject to error. The estimation of such an error is performed by the standard deviation s_{x_0}. Analyses of the simulated solution gave precise and accurate results, and were in good agreement with results of the reference method. If the range 90–110% of the stated concentration is considered to be an acceptable limit, three of the five brands examined were found to pass the analytical control and good agreement was noted with the alternative method. On the other hand, excessive or low thiomersal percentages recorded in samples D and E might be ascribed to preservative degradation and sorption taking place in the plastic container, or to incorporation of an excess of thiomersal by the manufacturer to counteract this process [4, 8, 12].

Of particular note is the presence of a significant amount of the bactericide chlorhexidine gluconate (5 mg 100 ml^{-1}) with thiomersal in product D. Nevertheless, this does not constitute a problem in the determination of thiomersal owing to both the different preconcentration potential (E_{acc} = 0 V) and the different peak potential (E_p = −1.53 V) required for the stripping voltammetric determination of chlorhexidine [13].

Conclusions

In comparison with the previously described polarographic techniques, AdSV, with its higher sensitivity and lower detection limit, allowed the rapid determination of thiomersal in contact lens solutions by simply adding into the voltammetric cell containing the supporting electrolyte (15 ml) a few microlitres of the commercial preparation, thus minimizing the possible interference of the other ingredients of the preparation. Besides, because of the ready photodegradation of thiomersal, that can lead to drastic reduction in the starting concentration (about 2.5 × 10^{-5} M) up to its complete depletion, AdSV appears to be a method of choice for preservative control in stability and storage studies of contact lens solutions.

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