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Short Communication

Semi-automatic analysis of mercury in pharmaceuticals by catalytic titration

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Introduction

The high toxicity of mercury, even at low concentrations, requires its determination in a great variety of samples in areas such as food and environmental analysis or clinical and pharmaceutical chemistry, by simple, sensitive and selective analytical techniques. A number of analytical techniques such as spectrophotometry [1, 2], cold-vapour [3–6] or electrothermal-atomization [7] atomic absorption spectrometry, X-ray fluorescence [8], neutron activation analysis [9] and voltametry [10]. Of these methods the spectrophotometric and atomic absorption techniques yield the best results in respect of sensitivity, precision and accuracy and have been used for the determination of mercury in pharmaceutical preparations.

The present paper describes a simple, sensitive and accurate method for the determination of mercury in pharmaceutical preparations; the method involves a recently developed mode of catalytic titration based on substrate inactivation [11]. In this catalytic titration mode the analyte–inhibitor interacts with the substrate instead of the catalyst. One of the reactants of the indicator reaction acts as titrant while the other and the catalyst are added to the titration vessel together with the analyte-inhibitor (mercury in this case).

Few kinetic methods for the determination of mercury have been proposed [12]; only one catalytic titration procedure has been reported for the determination of mercury in pharmaceutical preparations [13]. That method was based on the inhibitory effect of mercury on the iodide-catalyzed cerium(IV)–arsenic(III) reaction and involves monitoring the titration by potentiometry.

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Experimental

Reagents

4,4′-Dihydroxybenzophenone thiosemicarbazone was synthesized by condensation of 4,4′-dihydroxybenzophenone with thiosemicarbazide [14]. A 0.1% (m/v) solution of the reagent in ethanol was prepared. This solution was stable for at least one month. As the titrant, 25 ml of the solution was diluted to 250 ml with distilled water in a volumetric flask. This dilute solution was stable for at least four days.

A standard mercury(II) solution was made by dissolving 1.080 g of HgO in 20 ml of concentrated nitric acid and diluting to 1 l with distilled water. A standard copper(II) solution was prepared by dissolving the appropriate amount of $CuSO_4.5H_2O$ in water and was standardized iodimetrically. All dilute solutions were prepared just before use.

All other chemicals and solutions were of analytical reagent grade.

Apparatus

The complete instrumentation used for implementation of the semi-automatic photometric catalytic titration is depicted in Fig. 1. The equipment consisted of a Memotitrator (Mettler DL40) with an autoburette (10 ml), an independently operating, microprocessor-controlled compact instrument equipped with high-capacity storage facilities. As shown in Fig. 1, this instrument controls the instrumental variables in the catalytic titrations. A scanning phototitrator (Mettler DK18) with a dual-channel detector, filter system as monochromator and an immersion probe (Mettler DK181) which permits the measurement of absorbance or transmittance in the visible region was also used. The remainder of the instrumentation was a fan stirrer, the speed of which was measured by an electrical revolution sensor (IKA-TRON DZM1) in the range 5–500 rpm, a compact recorder (Mettler GA14) with a measuring range of 50–5000 mV and a chart drive operating between 1.6 and 20 mm ml^{-1} for a 10-ml burette, and a 20 cpl thermal printer (Mettler GA40).

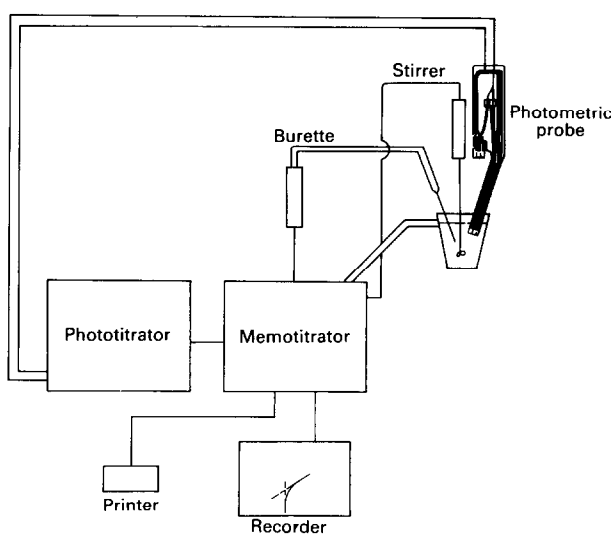

Figure 1
Schematic diagram of the semi-automatic photometric catalytic titration instrumentation.

Procedure

Direct semi-automatic catalytic titration of mercury. In a 100-ml titration vessel was placed a mixture containing 0.033–0.7 μM of mercury(II), 3 ml of 3.12×10^{-4} M copper(II), 2 ml of 0.15% (v/v) hydrogen peroxide solution and 4 ml of 1.5 M ammonia–0.45 M ammonium chloride buffer (pH 10.2); the mixture was diluted to about 80 ml with distilled water. This solution was stirred for 5 s and the titration started with 0.01% (m/v) reagent titrant solution at a speed of addition of 6 ml min^{-1}. The stirring speed was set at 200 rpm and the autocontrol system at position 1. The chart width was adjusted to 10 mm ml^{-1} and the initial absorbance was set at 0.200 (200 mV). From the titration curve of absorbance at 415 nm as a function of titrant volume, the end-point of the semi-automatic catalytic titration was determined graphically from the intercept of the straight line extrapolations before and after the equivalence point.

Results and Discussion

Ammoniacal solutions of 4,4′-dihydroxybenzophenone thiosemicarbazone (DBPT) are readily oxidized to a yellow-orange product by the action of hydrogen peroxide in the presence of copper(II), a catalyst for this indicator reaction. This catalyzed reaction has been used to determine submicrogram amounts of copper in water samples [15] as well as indium(III) and gallium(III) and mixtures of these species [16, 17] based on their activator effects, cyanide [18] and aminopolycarboxylic acids on the basis of inhibitory effects, both by kinetic methods [19] and by catalytic titrations [20].

Among the catalytic titrations proposed, the determination of metal ions based on an organic redox indicator reaction is usually performed by an indirect mode in which the catalyst acts as titrant [21–23]. In the present work, a different mode is used for the direct titration of mercury since this metal ion inhibits the oxidation of DBPT by hydrogen peroxide in the presence of copper. In this mode, known as catalytic titration by substrate inactivation [11], DBPT is used to titrate a solution containing mercury, the oxidant and the catalyst. At the start of the titration, the DBPT added is consumed through its complexation with mercury(II); once complexation is complete, the excess titrant starts the indicator reaction. The pseudo-induction period of the corresponding titration curves is directly proportional to the mercury(II) concentration in the sample (Fig. 2).

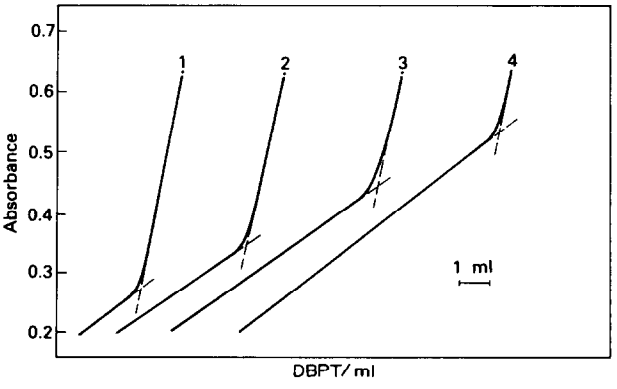

Figure 2
Titration curves for mercury(II): curves 1–4 correspond to 0.087, 0.26, 0.43 and 0.61 μM of mercury, respectively, under the recommended conditions.

The reaction between mercury(II) and DBPT has been studied recently [11] and the results obtained showed that it is not feasible to establish a clear stoichiometry for this complex since the mercury/DBPT stoichiometric ratio is influenced by time. This is attributable to the possible oxidation of DBPT to a phenoxy radical by an excess of mercury(II).

Consequently the proportional method, based on the stoichiometric relationship, cannot be applied to the determination of mercury. The influence of each variable (chemical and instrumental) was determined in order to obtain the maximum possible sensitivity (greatest slope) of the calibration graph. The pH of the titration solution has a marked influence on the reaction since above pH 10.2 the inhibitory effect of mercury(II) is considerably diminished; this can be attributed to the formation of hydroxo complexes of mercury.

Features of the analytical method

A linear calibration graph was obtained by plotting the titrant volume added (ml) against mercury(II) concentrations of 0.03–0.7 μM. The detection and quantification limits (0.01 and 0.033 μM, respectively) were calculated by multiplying by 3 or 10, respectively, the standard deviation of the added volume for 30 titrations of the same sample [24]. The upper limit of the calibration plot was limited by the volume of DBPT added in the titration, which cannot exceed 10 ml (i.e. the volume of the automatic burette used). The titration of 11 identical samples containing 0.43 μM Hg(II) gave a relative standard deviation of 1.9%.

Determination of mercury in pharmaceutical preparations

Heavy metals are all effective antibacterial agents because of their capability to react with proteins. Of special interest is the ability of ions such as Hg(II) to combine with the mercapto functions of some enzymes to form sulphides, thus blocking enzymatic action. Mercury chloride and other mercury compounds, both organic and inorganic (i.e. mercurials) have long been used as antiseptics and disinfectants. However, it has now been established that their activity is purely bacteriostatic, as confirmed for organic mercurials of more recent use.

Both soluble and insoluble inorganic mercurials are used in pharmaceutical preparations; organic mercurials include phenylmercury derivatives. The samples chosen for the experiments carried out to exemplify the proposed method were representative of all these types of mercurial. The proposed method is not specific insofar as it fails to distinguish between organic and inorganic mercurials because of the pretreatment of the sample that is required.

Sample preparation. Owing to its high content in the samples, organic matter had to be decomposed before analysis for mercury. Furthermore for greasy and soap preparations, such as sublimated soap and ophthalmic ointments, the fatty acids had to be removed by extraction with several 10-ml portions of diethyl ether in a nitric acid medium.

Destruction of the organic matter was carried out by a method based on the procedure recommended by the Analytical Methods Committee [25] by wet oxidation with $KMnO_4$–HNO_3. The original procedure was modified to make it suitable for the subsequent catalytic titration of mercury.

An appropriate amount or volume of pharmaceutical preparation was transferred to a 100-ml beaker and 2 ml of concentrated nitric acid was added followed by an excess of

3% (m/v) potassium permanganate solution. One 2-ml portion of concentrated nitric acid was added per 10 ml of potassium permanganate solution. The excess of permanganate was removed by adding 3% (m/v) sodium oxalate solution. In order to avoid the possible interference of manganese(II) in the subsequent titration, the mixture was made alkaline with ammonium chloride-ammonia buffer and several drops of concentrated hydrogen peroxide were added. The precipitate of manganese dioxide that was formed was removed by filtration and washed with several portions of hot water to recover the mercury. The filtrate was gently heated to remove excess hydrogen peroxide and diluted to 100 ml with distilled water in a volumetric flask.

Table 1 shows the amounts or volumes of treated samples as well as the aliquot taken in each case for the determination of mercury by the proposed catalytic titration procedure.

Determination of mercury. In accordance with the previously described procedures, the mercury content in seven pharmaceutical preparations was determined. Table 2 shows the results found by the proposed catalytic titration method compared with those obtained by the classical dithizone method [26]. It may be concluded that the results obtained by the catalytic titration method are consistent with those found by the dithizone method, although the former method is faster and simpler than the latter.

Table 1
Amount of sample taken and pretreatment applied in the determination of mercury in pharmaceutical preparations

Preparation	Sample	Pre-treatment	Aliquot (ml)
Sublimated soap	1.0642 g	Wet oxidation*	3.0
Mercury chloride lotion	25 ml	Wet oxidation	10.0
Ophthalmic ointment	0.1096 g	Wet oxidation*	3.0
Ophthalmic lotion	0.4 ml	—	
Exomycol	1.0250 g	Wet oxidation	5.0
Merthiolate	10 ml	Wet oxidation	1.0
Mercromina film	40 μl	Wet oxidation	3.0

* Prior extraction with diethyl ether.

Table 2
Determination of mercury in pharmaceutical preparations

Preparation	Mercurial present	Nominal content	Content found Catalytic titration*	Dithizone method
Sublimated soap	$HgCl_2$	0.20 g/100 g	0.206 ± 0.002 g/100 g	0.221 g/100 g
Mercuric chloride lotion	$HgCl_2$	1 mg/ml	0.98 ± 0.03 mg/ml	0.97 mg/ml
Ophthalmic ointment	$HgO + Hg_2Cl_2$	22.8 mg/g	21.6 ± 0.3 mg/g	22.3 mg/g
Ophthalmic lotion	HgI_2	5 mg/ml	5.00 ± 0.04 mg/ml	5.0 mg/ml
Exomycol	$C_6H_5Hg-H_2BO_3$	0.6 mg/g	0.616 ± 0.009 mg/g	0.619 mg/g
Merthiolate	Thiomersal	1 mg/ml	1.11 ± 0.01 mg/ml	1.14 mg/ml
Mercromina film	Merbromin	2 g/10 ml	1.97 ± 0.03 g/10 ml	2.16 g/10 ml

* Mean of five individual determinations.

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