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Amperometric quantification of sodium metabisulfite in pharmaceutical formulations utilizing tetraruthenated porphyrin film modified electrodes and batch injection analysis

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Abstract

The performance of a glassy carbon electrode modified with a porphyrin film formed by the $[Co(TPyP)\{Ru(bipy)_2Cl\}_4](TFMS)_5 \cdot H_2O$ complex for the analysis of sodium metabisulfite in pharmaceuticals is described. The sensor can be rapidly and easily prepared by drop-casting of a microliter volume of a diluted methanolic solution of the complex onto the electrode surface. The modified electrode with a supramolecular cobalt porphyrin film led to more favorable responses than the bare electrode. This can be ascribed to the much faster electron transfer processes to the analyte mediated by the tetraruthenated porphyrin and to the protection of the electrode against fouling. The association of the amperometric sensor with the batch injection analysis technique led to results that combine good repeatability of the current responses (relative standard deviation of 0.94% for 30 measurements), wide linear dynamic range (2.5×10^{-7} mol L^{-1} to 5.0×10^{-4} mol L^{-1}), high sensitivity and low limits of detection (8.1×10^{-8} mol L^{-1}) and quantification (2.7×10^{-7} mol L^{-1}). The system was successfully applied to sodium metabisulfite quantification in commercial samples of injection formulations of sodium (or potassium) diclofenac. The results compared well with those obtained by the polarographic method.
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Keywords: Tetraruthenated cobalt porphyrin; Modified electrode; Amperometry; Batch injection analysis; Sodium metabisulfite; Pharmaceutical products

1. Introduction

Sulfiting agents (sulfur dioxide and sodium or potassium sulfite, bisulfite and metabisulfite) have been widely used as preservatives, antioxidants and browning inhibitors in foods, beverages and pharmaceutical preparations. Among them, sodium (or potassium) metabisulfite has been commonly employed in oral, topical and parenteral medicines as an additive to avoid the oxidation of an active component. It is known that such compounds may cause allergic reactions in sulfite-sensitive persons. For this reason, the Food and Drug Administration (FDA) requires that the presence of sulfites (>10 ppm amounts) in processed foods must be declared in the label [1]. For drugs, FDA claims manufacturers to include

a label giving advices about all prescription drugs into which sulfites have been added. On the other hand, sulfites need not be inscribed on the labels of over-the-counter products. Thus, sensitive patients should contact the manufacturer to determine whether sulfites are used in specific over-the-counter products [2]. The symptoms related to allergic reactions vary from hives, nausea and diarrhea to bronchospasm (in asthmatic patients). The most frequent symptom is the difficulty in breathing leading to asthma attack. This is caused after sulfites give off the sulfur dioxide gas that brings about irritation in the lungs. The relation between asthmatic attacks and the use of sulfites has been well established through a sort of examining methods while reactions as hives or nausea are not yet proven [2]. Allergenic effects concerning administration of medicines containing sodium (or potassium) metabisulfite are not often documented, but there are several case reports of patients who were allergic to this sulfureous compound [3–5].

Methods for determining sulfitic species have been reported and are based on chemiluminescence [6–9], spectrophotometry [10–13], capillary electrophoresis [14–17] and electrochemistry [18–22]. Among the electrochemical techniques, the employment of chemically modified electrodes (CMEs) in analyses has demonstrated to be especially interesting owing to advantages such as sensitivity, selectivity and simplicity. Accordingly, many determination methods of sulfite using CMEs have been reported [18–22].

Sodium metabisulfite ($Na_2S_2O_5$) and potassium metabisulfite ($K_2S_2O_5$) have been one of the most commonly employed antioxidants in pharmaceutical formulations. In spite of this, only a few articles have been devoted to the quantification of metabisulfite in this kind of samples [17,23,24]. In this paper, we described an amperometric method for the analysis of sodium metabisulfite in commercial pharmaceutical preparations of sodium (or potassium) diclofenac by employing a glassy carbon electrode modified with films of a tetraruthenated cobalt-porphyrin (μ-{5,10,15,20-tetra(4-pyridyl)porphyrinatocobalt(III) }-tetrakis-{ chlorobis-(2,2′bipiridine)ruthenium(II)}) complex. In the last decade, we have reported successful applications of modified electrodes with films obtained by drop-casting or electropolymerization of tetraruthenated cobalt [22,25–28] and nickel [29] porphyrins. In the following sections, the determination of metabisulfite in injectable pharmaceuticals utilizing amperometry coupled to batch injection analysis (BIA) [30] is presented.

2. Experimental

2.1. Instruments and apparatus

The electrochemical experiments were carried out by using an EG&G Princeton Applied Research potentiostat/galvanostat (model 263A) interfaced with a microcomputer. Amperometry and cyclic voltammetry were performed with three-electrodes cell systems with capacity of 40 mL and 5 mL, respectively. The components utilized in both cell systems were a glassy carbon electrode (Model TL-5, Bioanalytical System) with 0.071-cm^2 apparent area as working electrode, a platinum auxiliary electrode and a miniaturized Ag/AgCl$_{KClsat}$ as reference. Amperometry was coupled to batch injection analysis utilizing the same arrangement previously described [27]. Injections of the solutions (standards and samples) were carried out with a motorized electronic micropipette EDP Plus EP-100 Rainin Instruments. Comparative polarographic measurements were obtained utilizing a Metrohm 646 VA Processor polarograph coupled with a 647 VA stand multi-mode mercury electrode. A polarographic cell with the working electrode in the dropping mode, a platinum counter-electrode and a reference electrode of Ag/AgCl$_{KClsat}$ was used in the experiments.

2.2. Reagents and solutions

The [Co(TPyP){Ru(bipy)$_2$Cl}$_4$](TFMS)$_5$·H$_2$O complex [25,27], or simply Co(TRP), was prepared by the reaction of Co(TPyP) with [Ru(bipy)$_2$Cl$_2$], in a molar ratio of 1:4. Analysis for C$_{125}$H$_{96}$N$_{24}$S$_5$O$_{19}$F$_{15}$Cl$_4$Ru$_4$Co, found (calcd.): C = 45.6 (46.2); H = 2.9 (3.1); N = 10.2 (10.3). Methanol, sodium metabisulfite, acetic acid, sodium acetate and sodium nitrate were of analytical grade and purchased from Merck. All solutions employed in the experiments were prepared with 18-MΩ ultra pure water from a Millipore® Milli-Q system. A new stock solution of sodium metabisulfite was daily prepared and suitable dilutions of standards and samples were performed just before the measurements.

Three different injectable pharmaceutical products of diclofenac containing sodium metabisulfite in their formulation were purchased in a local drugstore.

2.3. Modification of the electrode surface

The detailed procedure used for electrode surface modification was described elsewhere [27]. The glassy carbon electrode was polished with 0.3 μm and 0.02 μm alumina slurry on a felt cloth and then thoroughly washed with distilled water and dried at room temperature, before the deposition of the supramolecular modifying material. The CoTRP solution was prepared by dissolving 1 mg of the complex in 5 mL of methanol. One microliter of this solution was transferred onto a freshly cleaned surface and the solvent was allowed to evaporate. This procedure was repeated two more times in order to increase the thickness of the modifier film and thus to obtain more robust sensors.

2.4. Procedure

Experiments by cyclic voltammetry and amperometry were carried out with bare and modified electrodes in 0.2 mol L^{-1} sodium acetate/acetic acid buffer (pH 4.7) + 0.4 mol L^{-1} sodium nitrate solution, which was employed as supporting electrolyte. Typical conditions for cyclic voltammetry experiments were the following: initial potential, 0.0 V; scan rate, 10 mV s^{-1}; final potential, +1.0 V. For amperometric measurements, the working electrode was held at a constant potential of 0.7 V, which was very close to the value of the first current maximum observed in the voltammograms obtained for the modified electrode in the presence of sodium metabisulfite. Batch injection analysis experiments were made by injecting standard solutions (or samples) with different concentrations into the cell system that contained 40 mL of supporting electrolyte. The BIA parameters were varied in order to find the most suitable conditions for metabisulfite quantification. The best conditions found were the following: electrode–pipette tip distance, 2 mm; injected volume, 100 μL; injection speed, 75.3 μL s^{-1}.

Comparative measurements were carried out by dc-tast polarography in 0.1 mol L^{-1} sodium acetate/acetic acid (pH

4.7) buffer solution, using the multi-mode mercury electrode in the dropping condition. The experimental parameters after optimization were as follows: initial potential, −475 mV; sweep rate, 6 mV s^{-1}; final potential, −800 mV.

The potential values cited in this paper are referred to the Ag/AgCl$_{KClsat}$ electrode. All the experiments were performed at room temperature ($25 \pm 2\,^{\circ}$C).

2.5. Analytical conditions

In the amperometric analysis, sample aliquots from 40 μL to 150 μL were transferred to 10 mL volumetric flasks and the volume was completed with supporting electrolyte. In such a condition, a white precipitate occurred probably due to the formation of diclofenac acid. From this solution, aliquots of 400 μL of the supernatant were transferred to other volumetric flasks of 10 mL and the volume was completed with the same supporting electrolyte. The resultant solutions were analyzed by batch injection analysis–amperometry employing the calibration curve method.

For the polarografic measurements, aliquots of 100 μL (or 15 μL) of the samples were added to the cell containing 20 mL of 0.1 mol L^{-1} sodium acetate/acetic acid (pH 4.7), which was previously deoxygenated for more than 20 min and then the polarogram was obtained. After that, 20 μL aliquots of a 7.5×10^{-3} mol L^{-1} sodium metabisulfite standard stock solution were added and subsequent polarograms were recorded.

3. Results and discussion

3.1. Voltammetric studies

The electrochemical and electrocatalytic behavior of CoTRP films was discussed previously by our research group. When the [CoTPyP{Ru(bipy)$_2$Cl$_4$}]$^{5+}$ complex is dissolved in methanol and transferred onto glassy carbon electrode surface, adherent films are formed after solvent evaporation. In aqueous solution, the cyclic voltammograms of the modified electrode exhibit a pair of peaks in the 0.0–1.0 V potential range. The anodic ($I_{p,a}$) and cathodic ($I_{p,c}$) peak currents have nearly the same intensity and are separated by some millivolts, suggesting that the electrochemical process (attributed to the Ru(III)/Ru(II) redox couple) is almost ideally reversible. This is an indicative that the reaction kinetics is similar for the oxidation and reduction processes. In many cases, the porphyrin materials are soluble enough to undergo severe leaching by the electrolyte solution. During last 10 years, we have made successful efforts in order to find immobilization alternatives including the use of the counter-ions trifluoromethanesulfonate (TFMS) [25,26] or perchlorate [27] as supporting electrolyte and of electrostatically assembled films formed by alternating layers of cationic CoTRP and anionic *meso*-tetra(4-sulfonatephenyl)porphyrinate zinc(II) complexes [22,31,32] or more recently, the cationic *meso*(3-
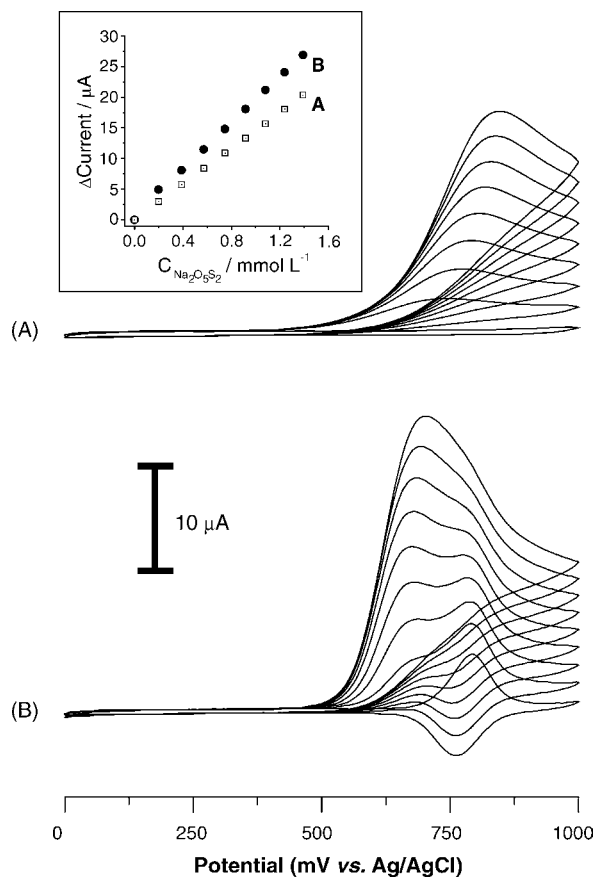

Fig. 1. Cyclic voltammograms of a bare (A) and CoTRP film modified (B) glassy carbon electrode immersed in 0.2 mol L^{-1} sodium acetate/acetic acid (pH 4.7) buffer + 0.4 mol L^{-1} sodium nitrate solution containing increasing concentrations of sodium metabisulfite (0 mol L^{-1} to 1.4×10^{-3} mol L^{-1}). Scan rate: 10 mV s^{-1}; electrode surface area: 0.071 cm^2. Inset: calibration plots obtained for solutions of sodium metabisulfite in the concentration range from 0 mol L^{-1} to 1.4×10^{-3} mol L^{-1} utilizing bare (□) and modified (●) electrodes.

or 4-pyridyl)porphyrins coordinated to four [Ru(bipy)$_2$Cl]$^+$, M(3-TRPyP) or M(4-TRPyP) (where M = 2H$^+$ or Zn^{2+}) was assembled with the anionic tetrasulfonated phthalocyaninate-cuprate(II) (CuTSPc) [33]. In present work, it was verified that glassy carbon electrodes modified with films of CoTRP also show good stability in sodium acetate/acetic acid buffer solution containing sodium nitrate, in special for measurements involving batch injection analysis. The peak currents in the voltammetric profile presented a decrease of only about 8% after 30 cycles. Therefore, this medium was employed in the experiments with CoTRP film modified electrodes, which was exploited for the determination of sodium metabisulfite in injectable pharmaceuticals.

The cyclic voltammograms recorded for the bare (A) and modified (B) glassy carbon electrode are compared in Fig. 1. For both systems, the potential sweep was done at 10 mV s^{-1} in 0.2 mol L^{-1} sodium acetate/acetic acid (pH 4.7) buffer + 0.4 mol L^{-1} sodium nitrate solution, before and after addition of sodium metabisulfite standards that

attained final concentrations from $2.0 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$ to $1.4 \times 10^{-3}\,\mathrm{mol\,L^{-1}}$.

The electrochemical process involving the oxidation of metabisulfite at the bare electrode was irreversible, with only a single oxidation wave in the potential range employed. The anodic peak potential shifted about 150 mV (from 670 mV to 840 mV) towards more positive values when the concentration of the analyte increased. This is an indicative that the electrochemical process is slow or yet that the electrode surface is being partially blockaded by the adsorption of the electrochemical reaction products.

In the presence of the porphyrin, a sharp anodic peak was observed and the peak potentials did not show significant shift. With successive additions of sodium metabisulfite led the current increased linearly as a function of the concentration. A similar behavior was found utilizing bare or modified electrodes; however, a more favorable current–concentration response was observed for the modified electrode (inset in Fig. 1). This can be ascribed to the faster electron transfer process involving the analyte and the ruthenated porphyrin.

3.2. Batch injection analysis

Experiments involving repetitive potential sweeps in the presence of metabisulfite showed a continuous decrease of the current signal when the bare electrode was employed. Contrary to this fact, the modified electrode exhibited a current response nearly constant as it was subjected to the same experimental conditions, suggesting that the porphyrin film also protects the electrode surface against fouling. This was confirmed in experiments in which amperometry was coupled to the batch injection analysis technique. The current signals obtained for successive injections of a $5.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ sodium metabisulfite solution using a modifier free electrode is shown in Fig. 2(A). A rapid and continuous decrease of the current can be seen, indicative of a loss of electrode activity probably due to the adsorption of the electrochemical reaction products as already mentioned above. On the other hand, injections of the same solution over the CoTRP film modified electrode showed very stable current signals (Fig. 2(B)). For this series of measurements, the relative standard deviation (R.S.D.) was calculated as 0.94% ($n = 30$), revealing thus the excellent stability of the sensor for multiple sequential analyses. The injections were carried out with a frequency of 60 determinations/h.

In order to find the most suitable experimental conditions for the metabisulfite quantification, some parameters involved in the BIA configuration were investigated. The best distance between the electrode surface and the pipette tip was 2 mm while 100 μL was the most appropriate sample volume. The fastest dispensing rate allowed in the programmable pipette ($75.3\,\mu\mathrm{L\,s^{-1}}$) was found to be the most suitable injection speed. The choice of the working potential (0.7 V) was based on the value correspondent to the first cur-
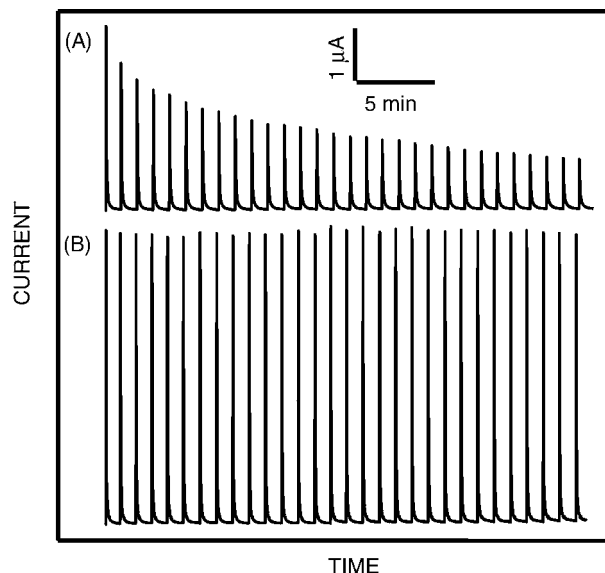

Fig. 2. Batch injection analysis results obtained for a bare (A) and CoTRP film modified (B) glassy carbon electrode for successive injections of a $5.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ sodium metabisulfite solution. Supporting electrolyte: $0.2\,\mathrm{mol\,L^{-1}}$ sodium acetate/acetic acid (pH 4.7) buffer + $0.4\,\mathrm{mol\,L^{-1}}$ sodium nitrate solution. Injected volume: 100 μL; dispensing rate: $75.3\,\mu\mathrm{L\,s^{-1}}$; working potential: 0.7 V; electrode surface area: 0.071 cm^2.

rent maximum observed on the voltammograms obtained for the modified electrode in the presence of metabisulfite (see Fig. 1(B)).

A series of experiments in triplicate was performed to get the analytical curves. A wide dynamic linear range was established for sodium metabisulfite concentrations, from $2.5 \times 10^{-7}\,\mathrm{mol\,L^{-1}}$ to $5.0 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$, with the calibration plot giving a correlation coefficient of 0.999. Higher concentrations than $5.0 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$ led to the deviation from the linearity as a result of a probable saturation of the system. A low limit of detection of $8.1 \times 10^{-8}\,\mathrm{mol\,L^{-1}}$ (three times the standard deviation of the blank [34]) was calculated utilizing a calibration plot in the $2.5 \times 10^{-7}\,\mathrm{mol\,L^{-1}}$ to $1.0 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ range. The limit of quantification was estimated as being $2.7 \times 10^{-7}\,\mathrm{mol\,L^{-1}}$ (10 times the standard deviation of the blank).

3.3. Analysis of the pharmaceutical samples

In order to check the potential usefulness of the sensor based on CoTRP films, such a modified electrode was exploited in analyses of sodium metabisulfite present in three different samples of injectable commercial preparations of sodium (or potassium) diclofenac. Before the injection in the BIA cell, the samples were conveniently diluted with the same supporting electrolyte present in the cell. Fig. 3 shows the results obtained for the sample 1, utilizing the calibration curve method. The excellent proportionality between the amperometric current and the concentration of the standard solutions (changing from $1.0 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ to $1.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$) can be easily confirmed in the inset,

Table 1
Experimental results obtained in the analysis of different commercial samples

Sample	Composition	BIA–amperometry ± S.D.[a] (mmol L^{-1})	Polarography ± S.D.[a] (mmol L^{-1})	Δ (%)
1	Sodium diclofenac, sodium metabisulfite, benzylic alcohol, mannitol, propyleneglycol, injection water, sodium hydroxide	3.45 ± 0.04	3.30 ± 0.04	4.3
2	Potassium diclofenac, sodium metabisulfite, benzylic alcohol, mannitol, propyleneglycol, disodic salt of the ethylenediamine tetracetic acid, injection water, potassium hydroxide	3.25 ± 0.13	3.32 ± 0.05	2.2
3	Sodium diclofenac, sodium metabisulfite, benzylic alcohol, mannitol, propyleneglycol, injection water, sodium hydroxide	12.33 ± 0.31	11.86 ± 0.23	3.8

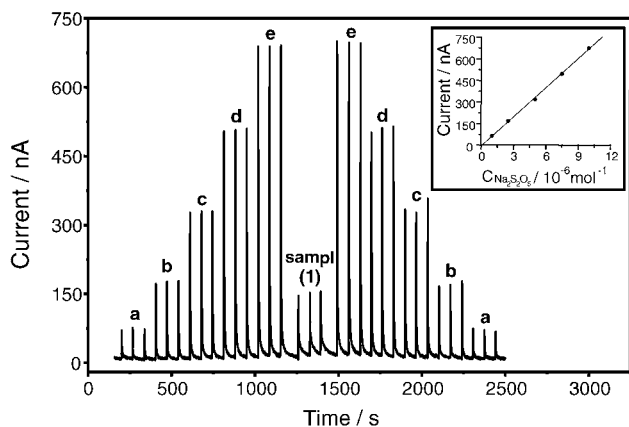[a] Average ± standard deviation of three determinations.

Fig. 3. BIA results for analysis of the sample 1 utilizing a glassy carbon electrode modified with a CoTRP film. Inset: calibration plot obtained after injections of: (a) 1.0×10^{-6} mol L^{-1}, (b) 2.5×10^{-6} mol L^{-1}, (c) 5.0×10^{-6} mol L^{-1}, (d) 7.5×10^{-6} mol L^{-1} and (e) $10 \times 10^{-6} \times 10^{-6}$ mol L^{-1} sodium metabisulfite standard solutions. Supporting electrolyte: 0.2 mol L^{-1} sodium acetate/acetic acid (pH 4.7) buffer + 0.4 mol L^{-1} sodium nitrate. Injected volume: $100\,\mu$L; dispensing rate: $75.3\,\mu$L s^{-1}; working potential: 0.7 V; electrode surface area: 0.071 cm^2.

where the calibration plot is illustrated. Also, it is possible to verify that even at such low concentrations, the base line was very stable and that the current responses showed a very favorable signal-to-noise ratio.

The results obtained by batch injection analysis and by dc-tast polarography are compared in Table 1, revealing relative differences ranging from 2.2% to 4.3%. Neither the active principle nor the other components that accompanied the formulations influenced the analyses. Calibration curves similar to that shown in Fig. 3 were obtained for the analyses of samples 2 and 3, utilizing the same CoTRP film. Only the solution in the BIA cell was renewed when the series of analyses of a new sample was started. The mean slope calculated from the three analytical curves was 65.6 mA L mol^{-1}, with a standard deviation of 1.5 mA L mol^{-1}, confirming the good stability of the sensor.

4. Conclusions

We have demonstrated the feasibility and applicability of amperometric sensors based on CoTRP films for analy-

ses of sodium metabisulfite in pharmaceuticals by associating the modified electrode with the batch injection analysis technique. The application of the method led to the rapid quantification of the analyte in injectable formulations of sodium (or potassium) diclofenac. The performance characteristics of the method demonstrate that it is suitable for analytical applications. Characteristics like good stability (R.S.D. of 0.94%), wide linear dynamic range (2.5×10^{-7} mol L^{-1} to 5.0×10^{-4} mol L^{-1}), high sensitivity and low limits of detection (8.1×10^{-8} mol L^{-1}) and quantification (2.7×10^{-7} mol L^{-1}) were achieved. The substitution of the toxic mercury electrodes by the more environmentally friendly modified electrode is also a worldwide tendency.

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