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Cloud point preconcentration prior to capillary zone electrophoresis: Simultaneous determination of platinum and palladium at trace levels

The incorporation of a cloud point extraction (CPE) step prior to capillary electrophoresis (CE) for simultaneously determining platinum and palladium at sub-μg/L levels is presented and evaluated. The analytes were extracted as 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complexes, at pH 2.0, mediated by micelles of the nonionic surfactant polyethyleneglycolmono-*p*-nonylphenyl ether (PONPE 7.5). The separation–determination step was developed from 150 μL of the extracted surfactant-rich phase diluted with 50 μL of acetonitrile (ACN). An exhaustive study of the variables affecting the cloud point extraction with PONPE 7.5 and the CZE step was done. The type and composition of the background electrolytes (BGEs) were investigated with respect to separation selectivity, reproducibility, and stability. A BGE of 50 mM monobasic sodium phosphate containing 30% ACN, pH 4.53 was found to be optimal for the separation of metal chelates. Detection was performed at 576 nm. An enrichment factor of 250 was obtained for the preconcentration of 50 mL of sample solution. The detection limits for the preconcentration of 50 mL of sample were 0.04 μg/L for Pt and 0.08 μg/L for Pd. As an analytical demonstration, ultratrace concentrations of platinum and palladium were conveniently quantitated in spiked water and urine samples.

Keywords: Capillary zone electrophoresis; Cloud point extraction; Palladium; Platinum
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1 Introduction

The determination of traces and ultratraces of platinum and palladium in the environment, as well as in body fluids of living species, at low concentrations is an urgent problem [1–5]. Pt and Pd affect the environment to an increasing degree as pollution, especially by the technical use of catalysts containing active Pt and Pd metals. The use of catalytic converters has led to an anomalous increase in the concentrations of these metals in several natural matrices such as soil, water, and vegetation, in areas near to intensive vehicle traffic, thus causing a new environmental risk [6–8]. A critical evaluation of possible risks for human health can only be given if reliable analytical data are available.

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Abbreviations: **5-Br-PADAP**, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; **CPE**, cloud point extraction; **PONPE 7.5**, polyethyleneglycolmono-*p*-nonylphenyl ether

The determination of platinum and palladium at low levels requires sensitive analytical techniques, for example, flame atomic absorption spectrometry (FAAS) [9, 10], electrothermal atomic absorption spectrometry (ETAAS) [6, 11, 12], inductively coupled plasma optical emission spectrometry (ICP-OES) [13, 14], inductively coupled plasma-mass spectrometry (ICP-MS) [15, 16], neutron activation analysis (NAA) [17], and capillary electrophoresis (CE) [18, 19].

Various methods have been developed for platinum and/or palladium separation and preconcentration from diverse matrices, such as anion exchange, precipitation, cloud point extraction (CPE), biosorption, coprecipitation, and sorption, among others [6–14, 17, 20].

During the past two decades, CE and related techniques have moved to the forefront of analytical chemistry [21] and have excelled in the rapid analysis of ions replacing traditional separation methods [22]. Metal analysis by CE has the advantages of robustness and ruggedness, low cost, rapidness, and versatility [23]. However, CE suffers from poor concentration sensitivity when using UV detection because of the small injection volumes (typi-

cally <1% capillary length) and narrow optical path length. This presents a significant obstacle for routine analyses of metal ions at ppb levels in real samples [24]. The need for enhancement of sensitivity has induced the development of highly sensitive detection and enrichment methods [25]. Nevertheless, approaches to enhance sensitivities are in most cases inadequate for metal analysis. In other cases, the low concentration levels, especially in environmental or clinical samples, are not compatible with the detection limits of these techniques. Consequently, preconcentration techniques in conjunction with CE represent a promising tool especially in the area of simultaneous determination of metals at subtrace levels [26–28]. Indeed, there is a continuing need for the development of alternative extraction/enrichment procedures which are safe, rapid, convenient, and accurate.

Surfactants are a special type of analytical reagent, which provides excellent improvements in almost every area of chemistry. In the last decades an increasing interest is shown all over the world in developing surfactant-based methods in all fields of analytical chemistry. At low surfactant concentrations above the critical micelle concentration, typically below 15 wt%, micellar solutions of nonionic surfactants can exist as homogeneous isotropic liquid phases. Phase separation can be induced in this concentration range by varying the temperature until the cloud point temperature is reached. Above the cloud point, the single isotropic micellar phase spontaneously separates into two phases, both of which contain surfactant but which differ in total surfactant concentration. In the small volume of the surfactant micellar-rich phase will be concentrated any components able to interact with the surfactant aggregate originally present in the sample [29–31]. From an analytical point of view, the surfactant-rich phase can be used to separate and/or preconcentrate different analytes before their injection into any hydrodynamic analytical system [30–32]. That is why CPE is particularly adequate for high-performance liquid chromatography (HPLC), flow injection analysis (FIA), and CE. Moreover, CPE uses surfactants that inhibit the absorption of nonpolar analytes to glass surfaces. The coupling of CPE to CE [33] and capillary electrochromatography (CEC) [34] has already been reported.

The aim of the present paper is to evaluate the feasibility of coupling a CPE preconcentration step to capillary zone electrophoresis (CZE) for the simultaneous determination of platinum and palladium at ultratrace levels. The preconcentration step is mediated by micelles of the nonionic surfactant polyethyleneglycolmono-*p*-nonylphenyl ether (PONPE 7.5) with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) as complexing reagent. The surfactant-rich phase was diluted with ACN. The factors that influence both the phase separation and extraction efficiency were critically assessed.

2 Materials and methods

2.1 Reagents and solutions

Platinum (IV) and palladium (II) standard solutions were prepared by appropriate dilutions of a 1000 mg/L stock solution (Fluka, Buchs, Switzerland) immediately before use. The solutions' pHs were adjusted with hydrochloric acid solution. The hydrochloric acid was Suprapure (Merck, Darmstadt, Germany). Hydrochloric acid (0.1 mol/L) was prepared by direct dilution with ultrapure water from the concentrated suprapure solution. A 1×10^{-2} mol/L solution of 5-Br-PADAP (Aldrich, Milwaukee, WI, USA) was prepared by appropriate dissolution in ethanol (Merck). As it is not possible to obtain a real aqueous solution of the surfactant PONPE-7.5 (Tokyo Kasei Industries, Chuo-Ku, Tokyo, Japan) (cloud point below room temperature), it was experimentally convenient to prepare a stock surfactant solution as follows: 20 g of PONPE 7.5, 10 mL of 1 mol/L $NaClO_4$ (Merck), and 40 mL of distilled ethanol were mixed and made up to 100 mL with doubly distilled water. The buffer solution (5×10^{-2} mol/L) was prepared by dissolving monobasic sodium phosphate (Merck) and made up to 1000 mL with ultrapure water. The BGE was prepared daily. Ultrapure water (resistivity 18.3 M$\Omega \times$ cm) was obtained from Barnstead EASY pure RF water system (Dubuque, IA, USA). All other reagents and solvents were of analytical-grade quality. All solutions were degassed by ultrasonication (Testlab, Argentina). Running electrolytes and samples were filtered through 0.45 μm Titan Syringe filters (Sri, Eaton Town, NJ, USA) and sonicated.

2.2 Instrumental

A Beckman P/ACE MDQ instrument (Beckman Instruments, Fullerton, CA, USA) equipped with a diode array detector and a data handling system comprising an IBM PC and P/ACE System MDQ Software (ESANCO) was used. Detection was performed at 576 nm. The fused-silica capillaries were obtained from MicroSolv Technology Corporation and had the following dimensions: 57 cm total length, 50 cm effective length, 75 μm ID, and 375 μm OD. The temperature of the capillary and the samples was maintained at 25°C. The pH of the electrolyte was measured by an Orion 940 pH meter equipped with a glass-combined electrode. All the glass instruments used were previously washed with a 10% v/v HNO_3/water solution and then with ultrapure water.

2.3 Experimental procedure

The water samples were filtered through 0.45 μm pore size membrane filters immediately after sampling, and were adjusted to pH 2.0 with hydrochloric acid solution and stored at 4°C in bottles (Nalgene; Nalge, Rochester, NY, USA). All the glassware and plasticware used were previously washed with a 10% v/v HNO_3/water solution and then with ultrapure water. Urine was collected and stored in plastic containers without adding preservatives. A sample mineralization was necessary since urine's natural occurring components normally do not allow Pt and Pd complexation with pyridylazo dyes. The samples of urine were digested as follows. A 50 mL sample of urine was accurately measured into a porcelain capsule, treated with a mixture of 2.0 mL of 30% w/w H_2O_2 and 1.0 mL 65% w/w HNO_3, and then placed in a sand bath. The sample was moderately heated up to cause disappearance of the amber color. Subsequently, the sample was evaporated to incipient dryness. Then, fresh portions of 65% w/w HNO_3 were added to the dark residue and heated to dryness. This procedure was repeated until a white ash was obtained. The residue was taken with 1.0 mL of 0.5 mol/L HCl. This solution was approximately diluted up to 50 mL with ultrapure water.

2.3.1 Preconcentration step

Fifty microliters of sample solution buffered to pH 2.0, 0.1 mL of 5-Br-PADAP reagent, and 0.5 mL of solution A was placed in a graduated centrifuge tube. This solution was kept at 50°C for 10 min for equilibration and then centrifuged for 5 min at 3500 rpm (1852.2 × *g*). After being cooled at −18°C for 5 min, the surfactant phase, which had separated, became a viscous gel and the aqueous phase could be poured off. A 150 μL aliquot of the surfactant-rich phase was transferred into the CE sample vial and diluted with 50 μL ACN.

2.3.2 Simultaneous determination of platinum and palladium by CZE

At the beginning of the day, the capillary was conditioned with 0.1 mol/L NaOH for 5 min, followed by water for 5 min, and then with running electrolyte for 10 min before sample injection. To achieve high reproducibility of migration times and to avoid solute adsorption, the capillary was washed between analyses with ethanol for 1 min, then sodium hydroxide for 2 min, followed by water for 2 min, and finally equilibrated with the running buffer for 4 min. Samples were pressure-injected at the anodic side at 0.5 psi for 3–7 s. To avoid buffer contamination caused by adsorption of surfactant onto the outer wall of the capillary, the anodic side of the capillary was immersed in ethanol for 2 s immediately after sample injection. A constant voltage was used for all the experiments. Detection was performed at 576 nm. EOF determination was performed by using acetone as an EOF marker. The EOF marker was prepared by diluting 1 mL of acetone with the BGE and sonication for 5 min prior to injection.

3 Results and discussion

3.1 Development of the preconcentration step

The effect of several experimental parameters upon the extraction parameters and sensitivity have been thoroughly evaluated and optimized. A summary of the optimal conditions is shown in Table 1.

The preconcentration conditions of the metal complexes were optimized and platinum and palladium signals were monitored by CE while changing the pH of the sample solution. The optimal pH values were in the range 1.5–3.0, in accordance with the optimal complex formation pH range. Considering these results, the selected pH was 2.0.

Table 1. Experimental optimized parameters

Cloud point preconcentration conditions	
pH	2.0
5-Br-PADAP concentration	2×10^{-5} mol/L
PONPE 7.5 concentration	0.1% w/w
Ethanol concentration	0.4% v/v
Equilibration temperature	50°C
Equilibration time	10 min
Centrifugation time	5 min
Cooling temperature	−18°C
Diluting agent	ACN
CE conditions	
Capillary	75 μm ID; 375 μm OD; total length 57 cm; effective length 50 cm
Cartridge temperature	25°C
Sample temperature	25°C
BGE	50 mM H_2NaPO_4/30% ACN; pH 4.53
Voltage	25 kV
Injection	Hydrodynamic, 5 s, 5 psi
Detection	576 nm

As regards the response variation with the molar concentration of the reagent 5-Br-PADAP, the analytical signal remained constant between 1×10^{-5} and 1×10^{-4} mol/L. A 2×10^{-5} mol/L 5-Br-PADAP concentration was adopted.

The effect of PONPE 7.5 concentration upon sensitivity and extraction parameters was studied within the surfactant concentration range 0.05–0.5% w/w. Quantitative Pd and Pt extractions were observed for an amphiphile concentration higher than 0.08% w/w. Results are shown in Fig. 1. In order to achieve a good preconcentration factor, 0.1% w/w was chosen as optimal.

3.2 Development of the separation conditions

In order to propose a specific and accurate way of analyzing CPE-preconcentrated water samples containing Pt(IV) and Pd(II), the following parameters were consecutively optimized: sample conditioning, pH, BGE composition and concentration, sample and capillary temperatures, and other electrophoretic parameters such as separation voltage, injection mode, and length, *etc.* (Table 1).

The effect of the buffer pH was investigated within the range of 2.5–6.0 at a fixed buffer concentration, adjusted by 0.1 mol/L NaOH and 0.1 mol/L HCl. It was found that when the pH was increased, resolution also increased, while time analysis decreased. The best results were obtained for a pH of 4.53.

Different BGEs have been tested, but the one producing the best results was monobasic sodium phosphate containing ACN, pH 4.53. Keeping other parameters constant (pH 4.53, 25 kV, 25°C) the buffer concentration was varied from 5 to 75 mM. Resolution also increased for higher buffer concentrations, but no appreciable improvements were observed for buffer concentrations above 50 mM. However, Pt and Pd were not completely separated.

ACN was used as an organic modifier to enhance the resolution. Various amounts of ACN (5, 10, 15, 20, 25, and 30% v/v) were added into the 50 mM monobasic sodium phosphate buffer, pH 4.53. The compounds were baseline separated when 30% v/v of ACN was added. So, a 50 mM monobasic sodium phosphate buffer containing 30% ACN, pH 4.53 was chosen as the BGE as it gave a full separation of the analytes of interest in less than 9 min. Separation of the analytes under optimal conditions is shown in Fig. 2.

The injection mode giving the best response concerning reproducibility and linear range was the hydrodynamic mode. Injection parameters were optimized by varying the lengths of sample (3–7 s) and pressure injection until

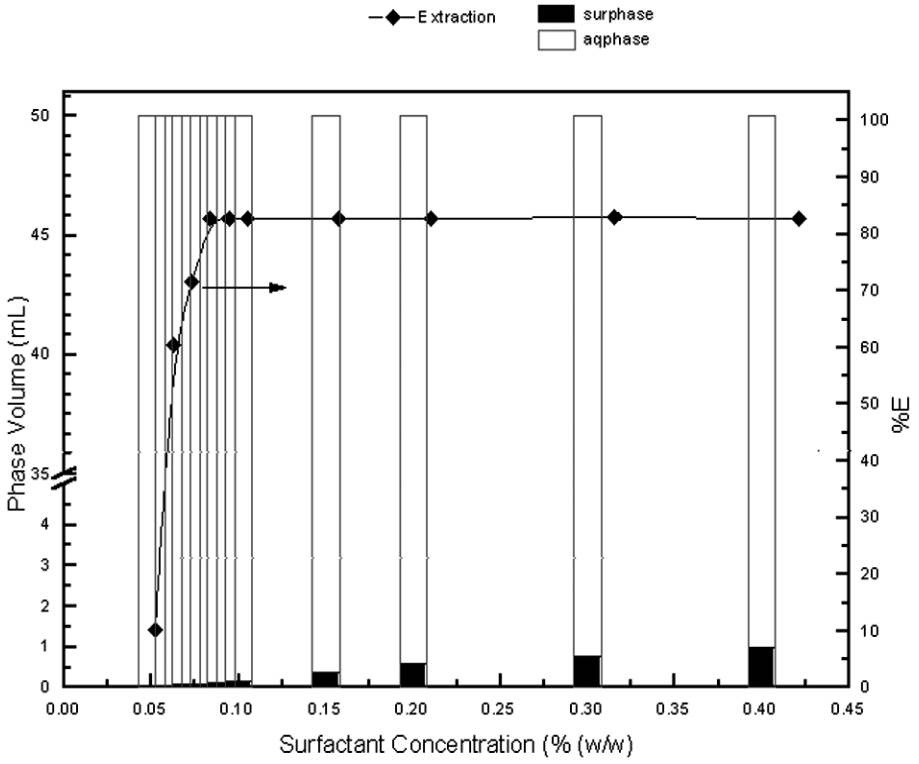

Figure 1. Effect of surfactant concentration. CPE conditions: pH = 2.0; 5-Br-PADAP concentration, 2×10^{-5} mol/L; equilibration temperature, 50°C; equilibration time, 10 min; centrifugation time, 5 min. Diluting agent: 50 μL ACN.

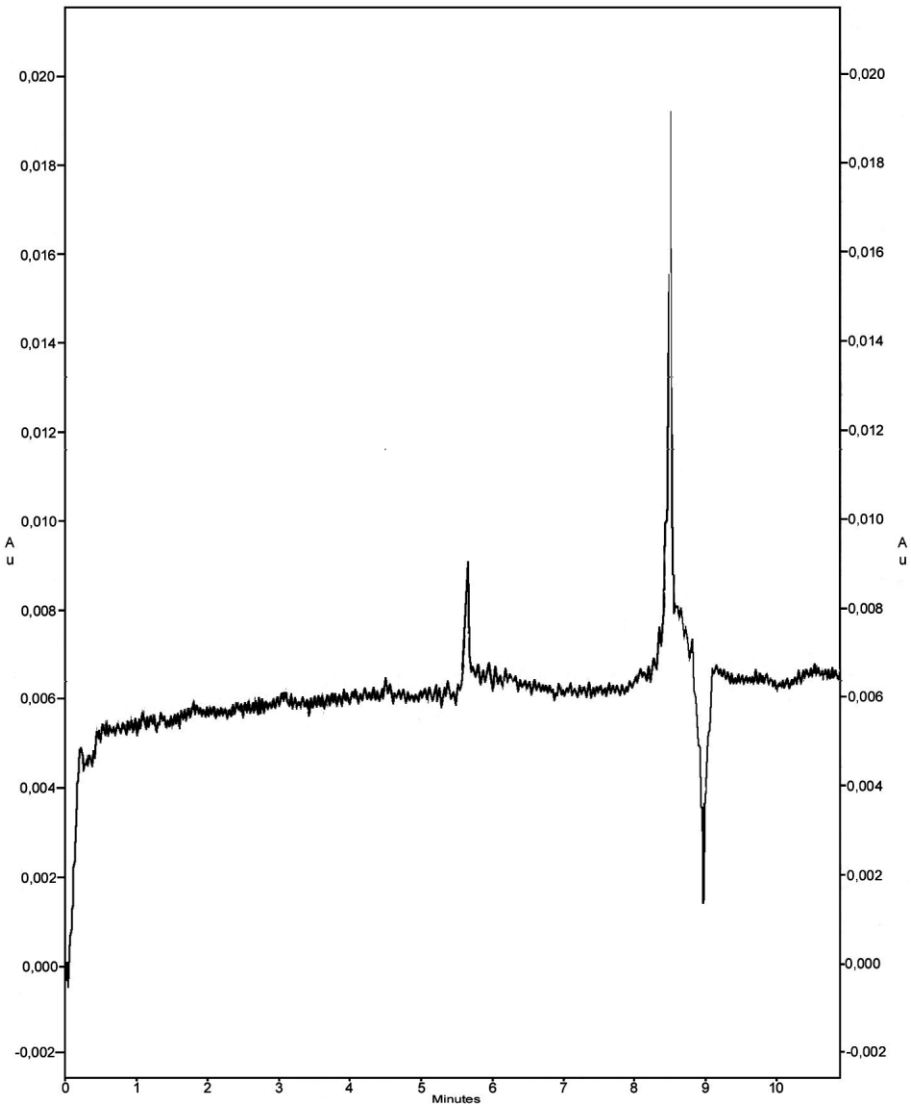

Figure 2. Electropherogram of a platinum–palladium spiked aqueous solution. Pt and Pd concentrations were 5 and 10 μg/L, respectively. CPE conditions as in Fig. 1. Separation conditions–50 mM monobasic sodium phosphate buffer containing 30% ACN, pH 4.53; capillary: 57 cm total length, 50 cm effective length, 75 μm id, 375 μm od; hydrodynamic injection mode: 0.5 psi, 5 s, 25 kV constant voltage; detection by direct UV-Vis absorbance at 576 nm.

optimum conditions were reached. The best results were obtained for the following experimental parameters: hydrodynamic injection mode 0.5 psi, 5 s.

Due to the high viscosity of the sample, buffer contamination caused by adsorption of surfactant onto the outer wall of the capillary was observed with consequent loss of separation efficiency and reproducibility. To avoid such effects, the anodic side of the capillary was immersed in ethanol for 2 s immediately after sample injection.

3.3 Interference studies

The preconcentration system proposed in this paper allows the elimination of most of the saline present in the samples, principally alkaline and alkaline-earth elements, due to the limited tendency of 5-Br-PADAP to form complexes with those elements under the experimental conditions. In addition, representative potential interfering species were tested. Thus, Cu^{2+}, Zn^{2+}, Pb^{2+}, Fe^{3+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, and Cr^{3+} could be tolerated up to at least 1000 μg/L.

3.4 Separation performance: evaluation of the combined methodology

Figure 2 shows the sample solution electropherogram obtained using the optimized experimental conditions. The migration times of Pt and Pd were found to be 8.91 and 5.62 min, respectively. Acetone was used as an EOF marker. These migration times did not vary to any

considerable degree during and in between analyses (%RSD less than 0.8 for the migration time of each peak and less than 2.4 for peak area). Resolution of Pt from Pd was 25.3. The analytes under study were baseline separated in less than 12 min, giving separation efficiencies of up to 359 029 average experimental electrophoretic plates (*N*).

An extraction percentage higher than 99.9% was achieved when the procedure was carried out under the optimal experimental conditions. Consequently, the enrichment factor achieved for this system was 250-fold.

The LODs of the analytes for the preconcentration of 50 mL sample volume, based on an S/N of 3 were 0.04 μg/L for Pt(IV), and 0.08 μg/L for Pd(II). This represents an excellent improvement in detection limits for the platinum group elements over previously published methods [18, 36–38]. The calibration graphs using the preconcentration system were linear with a correlation coefficient of 0.9997 (Pt) and 0.9994 (Pt) at levels near the detection limits up to at least 50 μg/L.

3.5 Method validation

As a certified value for Pt and Pd do not exist for any Certified Reference Material of Natural Water, the method of standard addition is considered as a validation method [35]; then in order to demonstrate the validity of this method, 500 mL of samples (tap water and human urine) were collected and divided into ten portions of 50 mL each. The proposed method was applied to six portions and the average quantities of Pd and Pt obtained were taken as a base values. Then, increasing quantities of platinum and palladium were added to the aliquots of sample, and platinum and palladium were determined by means of the preconcentration method. The results are given in Table 2.

4 Concluding remarks

In this report, a new coupled preconcentration/simultaneous determination approach based upon CPE in conjuction with CZE is described which could be applicable to many types of samples. This procedure has advantages over those currently available including low cost, safety, feasibility for simultaneous determination, and high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and very high concentration factors.

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Table 2. Recovery study (95% confidence level, $n = 6$)

Aliquots	Pt added, μg/L	Water sample		Urine sample	
		Pt found, μg/L	Recovery, %[a)]	Pt found, μg/L	Recovery, %[a)]
1–6	0.00	NF[b)]	–	NF[b)]	–
7	0.50	0.51 ± 0.04	102.0	0.49 ± 0.05	98.0
8	1.00	0.98 ± 0.04	98.0	1.02 ± 0.04	102.0
9	2.00	2.00 ± 0.03	100.0	1.98 ± 0.04	99.0
10	3.00	2.94 ± 0.05	98.0	2.98 ± 0.05	99.3
Aliquots	Pd added, μg/L	Pd found, μg/L	Recovery, %[a)]	Pd found, μg/L	Recovery, %[a)]
1–6	0.00	NF[b)]	–	NF[b)]	–
7	1.00	1.01 ± 0.04	101.0	1.01 ± 0.03	101.0
8	1.50	1.49 ± 0.03	99.3	1.51 ± 0.05	100.6
9	2.00	1.99 ± 0.03	99.5	1.98 ± 0.05	99.0
10	3.00	3.02 ± 0.04	100.7	2.99 ± 0.05	99.6

a) $100 \times$ [(found-base)/added]
b) NF: not found

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