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Review

Capillary electrophoresis of platinum-group elements Analytical, speciation and biochemical studies

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Abstract

A great deal of research has been carried out in recent years on developing high-efficiency capillary electrophoresis (CE) techniques that are able to separate rapidly and selectively ionic platinum metal species in a wide variety of their complexed forms. Using a range of illustrative examples, this review examines the potential and utilization of various CE separation approaches and detection modes in this expanding area. Also covered are CE procedures suitable for solving practical analytical problems and for platinum metal speciation purposes. Presenting a comprehensive treatise on the evolving practices of CE concerning platinum anticancer drugs—in particular, the examination of the stability of intact drugs, the separation and identification of products of their metabolism and interactions with biomolecules (including kinetic studies of the binding behavior)—this paper witnesses a welcome shift of the main research activities to those performed under physiological conditions.

Keywords: Reviews; Pharmaceutical analysis; Platinum-group elements; Metal complexes

Contents

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1. Introduction

Capillary electrophoresis (CE) has continued to mature as a useful tool for inorganic analysis in an ever expanding number of application areas. Owing to the technique's merits, such as an ability to provide impressive resolution even for complex mixtures of ionic species, high tolerance to complex matrices, rapid analysis, minor disturbances on the original distribution of element species, modest requirements on sample clean-up, etc., CE is particularly being used to an increasing extent for the separation and determination of platinum-group elements (PGEs) and their compounds. Indeed, whatever their chemical form, PGEs are chemically similar and hence closely migrating species. This feature might present a challenge for any separation method but least of all for CE. Though, often existing in solutions of high acidity or/and ligand concentration, PGEs can nonetheless be introduced directly into the separation capillary (provided that special adjustments in the carrier electrolyte composition have been done). This specific strength of CE simplifies an overall analytical procedure and becomes exceptionally significant for analyzing troublesome matrices, e.g. ores and alloys. Short analysis times allow the effect of possible chemical changes for a PGE in a CE system to be kept to a minimum and also make it a highly promising technique to study reaction kinetics. Furthermore, kinetic and stability studies related to biotransformations of platinum anticancer drugs can be carried out in the circumstances simulating physiological conditions since no organic solvent is commonly utilized in CE. The element speciation potential of CE is an important benefit because of a growing need in procedures for distinguishing and measuring individual PGE forms in environmental and biological matrices. On the other hand, taking into account the trace levels at which PGEs occur in such samples, certain problems should be emphasized as a consequence of inadequate detection sensitivity in CE, especially in comparison with high-performance liquid chromatography (HPLC). Only with the evolution of hyphenated methods, combining CE separation with very sensitive detectors, mainly inductively coupled plasma–mass spectrometry (ICP–MS), these problems appear to be solved.

The objective of this review is to demonstrate and discuss in more detail these and other significant advances as well as current limitations of CE as applied to PGE analysis. As no analogue issue has yet appeared in the literature, since a firstly traced published account of 1991 [1], a comprehensive coverage of contributions dealing with PGE separation by CE, is intended here. Also, in view of the authors' research interests, special emphasis will be placed on major developments in the speciation analysis of PGE and in studying platinum metal complexes customarily applied in cancer therapy or as promising anti-tumor agents by CE.

2. Separation of platinum metal complexes by CE

This section reviews migration modes and capillary electrolyte systems relevant to the separation of PGE complexes with different inorganic ligands. Also examined is the utility of organic complexing reagents that assist in electrophoretic separation of PGEs.

2.1. Inorganic–ligand complexes

Inorganic anions, mainly chloride and cyanide, comprise the group of complexing ligands most frequently used in CE of PGEs [2]. By virtue of the high ionic mobilities of the corresponding negatively charged complexes, they are typically separated by using a cathodic injection and anodic detection scheme following certain measures on reducing the magnitude of the electroosmotic flow (EOF) (see below). In a great many instances, no special capillary electrolyte additives are necessary to enhance the separation selectivity. This is due to sufficient differences in mobility provided by differences in charge-to-size ratio between different PGE complexes. On the other hand, an inclination to the stepwise dissociation accompanied by partial hydrolysis and the formation of hydroxo complexes and sometimes by oxidation–reduction reactions exhibits a feature of inorganic complexes undesirable for electrophoresis. These unfavorable effects inevitably require consideration when selecting optimized electrolyte conditions, and electrolytes of excessive ligand concentration serve almost always as the panacea. Detection of the PGE–inorganic ligand complexes, fairly strongly absorbing in the UV range, has been performed photometrically at wavelengths of a conventional UV detector (basically at 214 nm) or—in some speciation measurements—implementing ICP-MS (see Section 4).

Turning to the basic principles of CE regarding quantitative interpretation of the migration behavior of PGE complexes, a recent successful attempt to correlate the electrophoretic mobility of chloro and cyano complexes to their net charge (Z), the ligand number (n_L), metal atom electronegativity (or effective charge) and ionic radius (r_i) taken as variables of a multi-parametric migration model should be mentioned [3]. Consistent approximation results, as those shown, e.g. in Fig. 1, confirm the basic CE separation mechanism as governed by differences in charge density parameters of the analytes.

2.1.1. Chloro complexes

Chlorides received more literature coverage than complexes of any other single ligand. This is not surprising given the fact that chloro complexes originated from platinum metal standards or solid

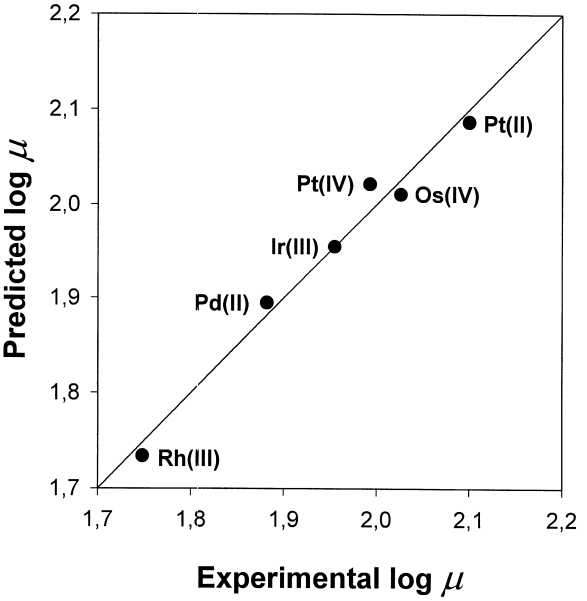


Fig. 1. Relationship between the migration parameters of PGE–chloro complexes determined experimentally [4] and calculated using equation $\log \mu = -0.306 + 1.206(\pm 0.391) \log Z + 1.805(\pm 1.939) \log \chi_M - 0.026(\pm 0.539) \log n_L - 6.495(\pm 2.910) \log r_i$. Slope 0.979, intercept 0.041, the correlation coefficient (R) is 0.9894.

samples are the common starting forms for the determination of PGEs (see Section 3). Carrier electrolytes based on a mixture of KCl (or NaCl) and HCl at pH 1.0–3.0 and total chloride concentration from 25 to 60 mM [4–7] up to 0.5 M [8,9] provide an environment where the complexes can be resolved, remaining essentially intact during a CE run. Also, at such acidic pH values and fairly high concentrations of electrolyte cations, the EOF is practically eliminated. This ensures reasonably short migration times in a primarily counter-electroosmotic migration mode. In order to speed up the migration of chloro complexes further, the electrophoresis conditions with reversed EOF ensured by adding a suitable long-chain cationic surfactant, namely cetyltrimethylammonium bromide (CTAB) [5,6,10], can alternatively be utilized. An increased ionic strength of the above electrolyte solutions does not notably affect the separation efficiency as thermal effects remain negligible [8–10] (unless the operating voltage is too high [7]), while narrowing of the peaks due to sample stacking becomes substantial [5,10]. In

the former study [10], chlorides of Pd(II) and Rh(III) were specifically used as test solutes to detect mutual relationships between applied voltage, migration time and peak area.

Working with the best selections of CE conditions yields a complete resolution of up to five-component mixtures of different PGEs as well as Au(III) [4,7]. However, up to now the utmost separation performance, i.e. all six PGEs separated in one run, is still missing. Fig. 2 demonstrates perhaps the most advantageous—in terms of the multi-elemental ability and analysis time—example achieved in the work by Pirogov and Havel [4].

2.1.2. Cyanides

The research interest in cyano complexes is imposed by an extensive use of cyanide in the precious metal mining, processing and recovery industries. In general, moderately alkaline electrolyte buffers offered a rapid simultaneous separation of cyano complexes of Pt(II) and Pd(II) along with those of Au, Ag and a number of nonplatinum group metals [11–14]. Dynamic treatment of the fused-silica capillary wall with hexamethonium bromide [12,13] facilitated a faster migration, by reducing to a certain extent EOF, affecting the resolution via ion-pairing effects. The electrolyte additives influencing separation selectivity and analysis time also encompass organic solvents [12,14], perchlorate [13], short-chain quaternary ammonium salts [15] as well as free cyanide [15]. For instance, the baseline resolution Pd(II)- and Pt(II)-cyano complexes in a co-electroosmotic system based on the use of a phosphate buffer at pH 11 and tetradecyltrimethylammonium bromide (TTAB) as an EOF-modifier, was only feasible in the presence ion-pairing tetrabutylammonium bromide (TBAB) and NaCN that favors the formation of cyano complexes with a higher number of ligands [15]. An electropherogram shown in Fig. 3 is the separation obtained under optimized concentration of cyanide and perchlorate in the carrier electrolyte [13]. Note that cyanide-free electrolytes are used when determination of PGEs is the task but are not suitable for quantifying metallo-cyanides in, e.g. environmental samples.

2.1.3. Miscellaneous

Among alternate inorganic ligands, bromide and thiocyanate have been explored for separating PGEs

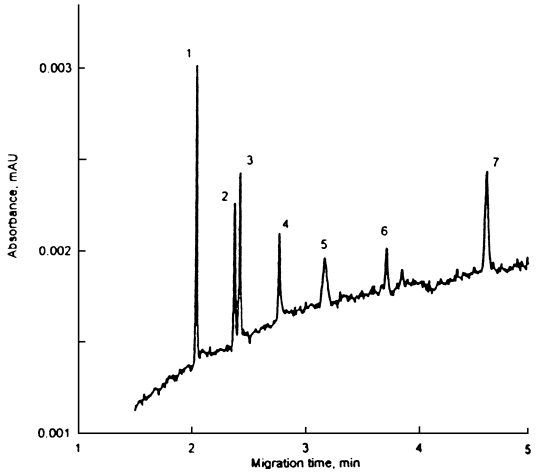


Fig. 2. Separation of platinum group metals as chloro complexes. Conditions: capillary, fused-silica, 75 μm×44.5 cm I.D.; electrolyte, 60 m*M* KCl (pH 3.1); temperature, 55 °C; sample introduction, electromigration (2 s at 5 kV); voltage, −11 kV; direct UV detection at 214 nm. Peaks (mg l^{-1}): (1) Pt(II) (10); (2) Os(IV) (3); (3) Pt(IV) (2); (4) Ir(III) (5); (5) Pd(II) (4); (6) Pt(IV) (hydrolysis product); (7) Rh(III) (2). (Reprinted with permission from Ref. [4]).

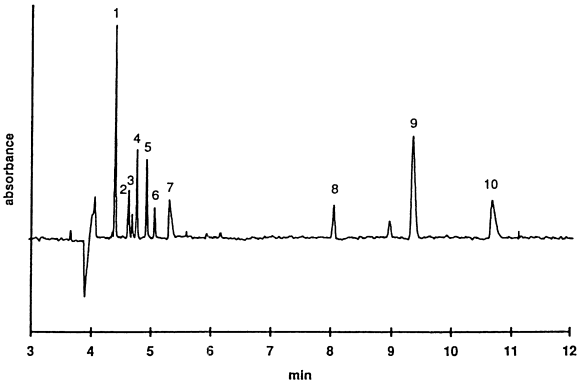


Fig. 3. CE separation of ten precious and transition metallocyanides. Conditions: capillary, fused-silica, 75 μm×60 cm I.D.; electrolyte, 5 m*M* Na_2HPO_4, 5 m*M* triethnolamine, 15 m*M* $NaClO_4$, 0.8 m*M* hexamethonium bromide, 0.1 m*M* NaCN (pH 8.5); sample introduction, gravity (10 s at 10 cm); voltage, −25 kV; direct UV detection at 214 nm. Peak identification: (1) $Fe(CN)_6^{4-}$; (2) $Co(CN)_6^{3-}$; (3) $Fe(CN)_6^{3-}$; (4) $Ni(CN)_4^{2-}$; (5) $Pd(CN)_6^{2-}$; (6) $Pt(CN)_4^{2-}$; (7) $Cu(CN)_4^{3-}$; (8) $Cr(CN)_6^{3-}$; (9) $Au(CN)_2^-$; (10) $Ag(CN)_2^-$. (Reprinted with permission from Ref. [13]).

but with less success compared to that attained by using chloride and cyanide. The bromide complexes of Pd(II), Pt(IV), Ir(IV), Rh(III) and Au(III) were separated in an bromide-containing electrolyte of pH 3.0 [16]; however, their complete resolution took about 50 min. Lower stability and greater relative size of PGE-bromides are the reasons for the worsened electrophoresis behavior. The same mode of migration and a similar acidic ligand-free electrolyte enabled CE separation of palladium(II) and platinum(IV) in the form of thiocyano complexes [17]. Other PGEs have not been tried except for Pt(II) which forms the thiocyanate complex with quite a different absorbance maximum and therefore can be determined only individually.

2.2. Complexes with organic ligands

A large proportion of work, dealing with the determination of PGEs through CE separation, involve complexation prior to electrophoresis by using organic complexing reagents. This is due mainly to their advantages: offering very sensitive photometric detection and preserving PGE species against any chemical transformation during CE. Depending on their complexation functionalities and the presence (or absence) of functional groups taking no part in complexation, organic reagents form with PGEs anionic, cationic or neutral complexes which are considered separately in the following subsections.

2.2.1. Anionic chelate complexes

There exists a diversity of chelating reagents capable of converting PGE into negatively charged complexes. Of these chelators, aminopolycarboxylic acids [18–21], heterocyclic azo dyes [22,23], nitrosonaphtholsulfonic acids [24–26] and some others like 5,10,15,20-tetrakis(4-sulfophenyl)porphine (TPPS) [27] and 2,6-pyridinedicarboxylic acid [28] have found an application in CE. However, with the only exception [23], PGE complexes of this type are limited to Pd(II) chelates. On the other hand, along with palladium a great number of other group metals (up to 20) can be separated simultaneously and this opens up the possibility for selective determination in various practical samples.

Separation of relatively slow migrating anionic PGE complexes are most customarily performed in an anodic injection and cathodic detection CE mode with neutral to moderately alkaline buffer electrolytes, containing a micromolar (or lower) concentration of a free ligand to prevent in-capillary dissociation of the complexes. Under such conditions, the EOF velocity is high enough to allow the complexes to be carried fast in the direction opposite to their electrophoretic movement. To finetune the resolution, some electrolyte compositional changes might be optional, and an organic solvent such as ethylene glycol for the complexes of *trans*-1,2-cyclohexanediaminetetraacetic acid [18,21] and hydrophobic ammonium compounds are the most viable additives of choice. For instance, a recent report by Liu et al. [23] demonstrated the advantage of using water–ethanol electrolytes for the separation of Ru(III), Rh(III) and Os(IV) in the form of 4-(2-thiazolylazo)resorcinol (TAR) complexes. As can be seen in Fig. 4, Os(IV) migrates as a positively charged chelate, confirming the use of the CE method for simultaneous analysis of cationic and anionic species. The TBA cation added to the electrophoretic buffer induced drastic changes in mobilities and improved peak shapes for anionic chelates of 2-nitroso-1-naphthol-5-sulfonic acid [24,25] and 1-nitroso-2,7-dihydroxynaphthalene-3,6-disulfonic acid [26] as a result of (selective) ion association. Note that although the potential of precapillary derivatization regarding sensitivity of direct photometric detection is recognized as especially encouraging with visible light-absorbing metallochromic reagents, detection limits lower than $10^{-6}M$ are exceptionally accessible for PGEs [27].

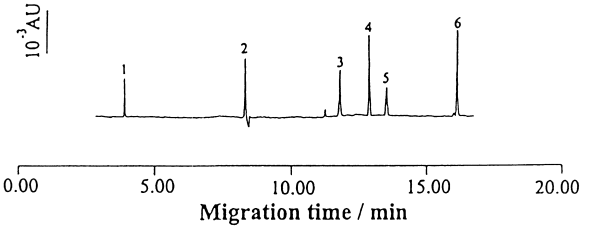


Fig. 4. Electropherogram of platinum metal–TAR complexes. Conditions: capillary, fused-silica, 50 μm×70 cm I.D.; electrolyte, 10 m*M* NaH_2PO_4–Na_2HPO_4, 1 m*M* TAR, 10% (v/v) ethanol (pH 6.5); sample introduction, vacuum; voltage, 30 kV; direct photometric detection at 540 nm. Peaks: (1) Os(IV); (2) neutral impurity (EOF); (3) Rh(III); (4) Co(II); (5) Ru(III); (6) TAR. (Reprinted with permission from Ref. [23]).

2.2.2. Cationic complexes

To the authors' knowledge, there are only four reports on CE separation of PGEs following precapillary transformation into cationic complexes. Thiourea, a well-known complex-forming reagent for precious metals, enabled a fast separation of Rh(III) from Pd(II) or Pt(II) but all efforts to resolve the latter two PGEs proved unsuccessful [29]. 2,2′-Bipyridine (bpy) proved to be suitable for the selective resolution of palladium(II) from a group of transition metals in an electrolyte system containing CTAB apparently to decelerate the EOF [30]. In specific studies by Kane–Maguire [31], Wheeler [32] and co-workers, the determination of enatiomeric purity of Ru(II) complexes with α-diimine ligands (1,10-phenanthroline, bpy, etc.) was achieved using antimonyl-*d*-tartrate or dibenzoyl-L-tartrate anions in the electrophoretic buffer for selective ion-pairing and hence discriminating the optical isomers. The respective Δ isomer showed a greater interaction with the chiral additive and thereby a slower mobility. As a result, baseline separation of a series of four chiral *rac*-$[Ru(diimine)_3]^{2+}$ compounds into their eight respective isomers was remarkably achieved [32].

Summarizing, more research is anticipated on the CE method of positively charged PGE complexes exhibiting short separation times owing to co-electroosmotic migration thereby implying the same direction of electrophoretic and electroosmotic flow.

2.2.3. Neutral chelates

A few studies have involved uncharged PGE chelates amenable to separation by micellar electrokinetic chromatography (MEKC) (see Ref. [33] for their partial overview). To introduce the MEKC conditions, the addition of a negatively charged surfactant, sodium dodecylsulfate (SDS) at above the critical micellar concentration is the one and only step. In such a way, Pd(II), Pt(II) and Rh(III) were efficiently discriminated through the differential interaction with a SDS micellar pseudostationary phase [1]. Prediction of such separations rest upon liquid–liquid partition data since the distribution coefficient of each metal complex between the micelle and the electrolyte buffer solution, calculated directly from the capacity factor k', was found to be linearly related to the partition coefficient (P) for a dodecane–water system. In a recent theoretical study by one of the authors [34], a more straightforward relationship between migration time (t_m) and P was established, as shown in Fig. 5. Haddad and co-workers in three successive works [35–37] pursued bis(2-hydroxyethyl)dithiocarbamate (HEDTC) as a complexing reagent for MEKC separation of a number of transition metals, including Pt(II). Excellent separation efficiencies typical of MEKC provided this PGE with the lowest concentration detection limit ever observed with photometric detection, i.e. 3.6×10^{-8} *M*, which is only about seven times above that of CE with ICP-MS detection. Also, a valid migration model was developed to relate the effective mobility of the HEDTC complex to the coefficient of its partition into the micelle, electrophoretic mobility and variables of the electrolyte solution such as concentrations of SDS and methanol [37]. Using nonlinear regression, parameters of the model were derived from experimental data and then used to predict effective mobilities of the analytes, yielding results in fairly good agreement with observed mobilities.

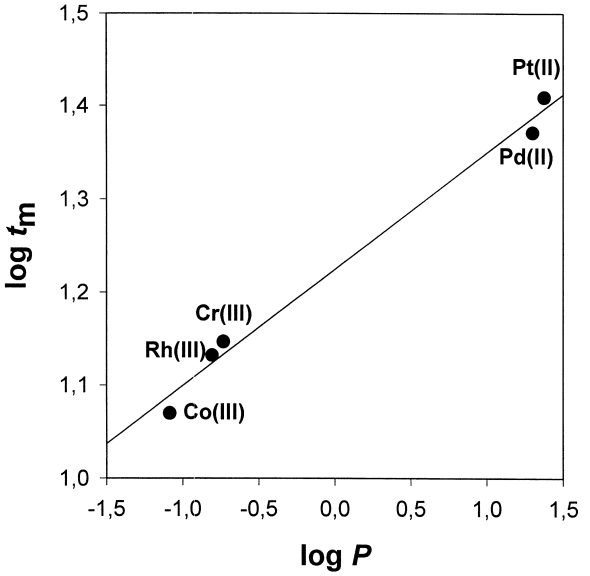


Fig. 5. Log t_m − log P correlation plot for metal–acetylacetonato complexes. Correlation coefficient, 0.9946.

3. Determination of platinum metals in practical samples

A range of aforementioned CE systems have been evolved for solving real-world PGE analysis problems. However, despite constantly growing anthropogenic emissions of PGEs, concentrations of these metals found in environmental samples still remain rather low and far beyond the detection limits reached by current CE methodology. Therefore, samples with relatively high concentration levels of PGEs are only amenable to direct analysis. Among such samples, certain industrial and pharmaceutical matrices have attracted most of the attention since these are catalytic converters for motor vehicles and platinum based anti-tumor drugs that represent the main sources of PGE released into the environment. Table 1 gives an inclusive listing of applications related to the determination of total PGE concentrations by CE. For a reference of readers interested in the determination of precious metals, a few contributions that deal with quantifying silver and gold are also included. Also to be mentioned in this section is a specific application of PGE complexes, namely $Ru(bpy)_3^{3+}$, for chemiluminescence detection of various analytes of biochemical interest in CE [40].

3.1. Solid samples

Evidently, the PGEs of interest should first be released from a solid matrix by using an efficient leaching procedure; otherwise, complete sample dissolution must be employed. Both steps while implying rigorous conditions (i.e. treatment with strong complexing agents or mineral acids at high temperature) require a prolonged time to ensure complete recovery. This negates the technique's benefit of short analysis times and also calls for prompt attention to be paid to the development of more efficient sample pretreatment procedures. In this context, closed decomposition vessels [15,17], which enhance the rate of decomposition and reduce the risk of contamination and losses, deserve implementation.

A CE method for the determination of Pd(II) and Pt(II) as well as Cu(I), Fe(II) and Ni(II) cyano complexes has been tailored to the analysis of cyanide leaching solutions from automobile catalytic converters [15]. Adsorption onto activated charcoal as a means of recovering the PGEs from the leached solutions has also been studied by this method (Fig. 6). For both leaching and adsorbed samples results were similar to those of ICP atomic emission spectroscopy (ICP-AES). To extend the potential of this and analogue procedures to environmental applications, pre-electrophoresis enrichment of PGE–cyanide complexes is necessary, and both solid-phase [12] or supported liquid membrane [13] extraction appear promising, resulting in a considerable improvement of the detection limits. Similar converter samples were analyzed after wet digestion by aqua regia [9]. The use of high chloride electrolyte (see Table 1) permitted the resulting solution to be introduced into the capillary—following an appropriate dilution—without impairment of the CE system performance. Other solid samples analyzed by CE involve a palladium catalyst [27], standard material of vehicle exhaust particulates [17], anode slime obtained from an electrolytic copper refinery [14], gold and silver ores [38,39].

3.2. Aquatic samples

As follows from Table 1, much of the work in the preparation of liquid samples had to be spent on bringing the PGEs into a complexed form (chloro, bromo, thiocyano, etc.) suitable for subsequent CE analysis. While making the analytical scheme more tedious, such treatments do not seem to affect the quality of analysis. Accordant accuracy assessments via comparing the results obtained with the CE and reference method, namely ICP-AES [4] and ICP-MS [17], or agreement with certified values [16,17,29] were reported.

A series of anticancer chemotherapy drugs based on platinum complexes (see Section 5) have been assessed for the total Pt content by Havel and coworkers [4,17]. The CE method also provides an efficient means for the analysis of various technological [4,29] and standard [16,29] solutions. For example, Fig. 7 illustrates the possibility of the quantification of Pd, Pt, Ir, Rh and Au from a multi-element standard that comprises all of the PGEs along with many other elements [16]; osmium and ruthenium underwent distillation in the form of

Table 1
Summary of real-world applications

Sample	Sample preparation	PGE determined (LOD)[a]	CE conditions[b] / UV detection wavelength (nm)	Ref.
Catalytic converter (spent)	Dissolution in aqua regia for 12 h, dilution	Pt(IV) (1.4 mg l^{-1})	0.4 *M* NaCl, 0.1 *M* HCl/270	[9]
Catalytic converters (virgin and used)	Leaching with a 10 g l^{-1} NaCN solution in 0.1 *M* NaOH in an autoclave for 1 h at 140 °C, decantation of the leaching solution, centrifugation, filtration	Pd(II) (53 μg l^{-1}), Pt(II) (18 μg l^{-1})	20 m*M* Na_2HPO_4, 20 m*M* NaCl, 100 m*M* NaCN, 3 m*M* TBAB, 40 μ*M* TTAB, pH 11.0/208	[15]
Palladium asbestos	Treatment with aqua regia for 15 min at 100 °C, filtration, addition of conc. HCl, drying, dissolution of the residue in 0.01 *M* HCl, addition of acetate buffer (pH 4.5) and a TPPS solution, heating for 15 min at 100 °C	Pd(II) (0.8 μg l^{-1})	100 m*M* citrate buffer, pH 3.5/410	[27]
Vehicle exhaust particulates	Combustion in a plasma mineralization apparatus for 5 h, addition of conc. $HClO_4$, drying, dissolution of the residue in water, filtration	Pd(II) (0.3 mg l^{-1})	100 m*M* KSCN, pH 3.0/305	[17]
Ores	Leaching by shaking with an alkaline solution of 0.01 *M* NaCN for 100 h, centrifugation	Ag(I) (3.5 mg l^{-1}), Au(I) (2.8 mg l^{-1})	10 m*M* carbonate buffer, pH 9.6/214	[38]
Ores	Leaching with an alkaline solution of 1 g l^{-1} NaCN for 24–48 h, centrifugation, filtration	Ag(I) (16 μg l^{-1}), Au(I) (80 μg l^{-1})	20 m*M* Na_2HPO_4, 36 m*M* NaCl, 1 m*M* NaCN, 10–30 μ*M* TTAB, pH 11.0/204	[39]
Anode slime	See the paper	Pd(II), Pt(II)	30 m*M* phosphate buffer (pH 7.0), 15% (v/v) acetonitrile/214	[14]
Multi-element standard solution	Addition of aqua regia, evaporation to near dryness, addition of $HClO_4$ (1:1) and NaBr, evaporation, dissolution of the residue in $HClO_4$, evaporation, dissolution of the residue in conc. HBr, adjustment of the pH to 3.0, addition of acetate buffer of pH 3.0, dilution	Ir(IV), Pd(II), Pt(IV), Rh(II), Au(III)	50 m*M* CH_3COONa, 30 m*M* $HClO_4$, 50 m*M* NaBr, pH 3.0/235	[16]
AAS standard	Addition of 70% $HClO_4$ (on heating) and 1 *M* thiourea, dilution, heating for 60 min at 100 °C	Rh(III) (40 μg l^{-1})	20 m*M* $NaClO_4$, 1 m*M* thiourea, pH 2.5/300	[29]

Table 1. Continued

Sample	Sample preparation	PGE determined (LOD)[a]	CE conditions[b] / UV detection wavelength (nm)	Ref.
Platination solution	Dilution ($1:10^3$)	Pt(II)	60 m*M* KCl, 1 m*M* HCl, pH 3.1/214	[4]
Platination solution	Dilution ($1:10^4$), treatment as described above under Ref. [29]	Pt(II) (20 $\mu g\ l^{-1}$)	20 m*M* $NaClO_4$, 1 m*M* thiourea, pH 2.5/275	[29]
Carboplatin-containing drug	Addition of aqua regia, evaporation to dryness, dissolution of the residue in 0.2 *M* HCl	Pt(IV) (0.2 $mg\ l^{-1}$)	60 m*M* KCl, 1 m*M* HCl, pH 3.1/214	[4]
Cisplatin- and carboplatin-containing drugs	Dilution (1:100) of the drug solution, addition of 1 *M* KSCN, heating for 7–10 min at 100 °C, dilution	Pt(II) (0.9 $mg\ l^{-1}$)	100 m*M* KSCN, pH 3.0/245	[17]

[a] Detection limit where available.
[b] Untreated fused-silica capillary.

bromides during the sample pretreatment and therefore could not be subject to analysis.

4. Analysis of platinum metal species

CE studies on distinguishing different species of one PGE have grown in significance during the past few years [41,42]. On the one hand, knowledge about the chemical form in which a PGE occurs is of immense importance when assessing how serious contamination of the environment and the risk associated with PGE pollution. As the PGEs species are often susceptible to numerous chemical transformations, there is a likelihood that at least some of the species discharged into the environment and formed during transformation into its different components can become bioavailable. This allows uptake by plants and thus opens a path into the food chain. On the other hand, CE procedures for measuring individual forms of PRE combine a capacity to discriminate a speciation pattern on the basis of subtle charge-to-size differences, short analysis time, a small number of operations involved in sample pretreatment, the minor influence of a CE system on the equilibrium pre-existing in the sample, etc. These merits make CE compatible with other analytical techniques capable to provide direct information about the speciation of PGEs such as HPLC [43–45].

4.1. Platinum species

Most investigations of PGE speciation by use of CE have focused on platinum [4,7,9,46–48]. This listing does not include a few studies concerning real samples which are considered separately (see Section 4.3). Platinum is recognized as the environmentally most relevant PGE, comprising: (i) approximately 85% of the active metals in modern three-way catalysts; (ii) the only PGE that so far found an application to cure cancer diseases; and (iii) a substantial part of amalgam teeth fillings. It should be noted that contributions on the platinum complexes, products of their metabolism and interactions with biological systems are the subject of our later discussion (Section 5).

As mentioned above, PGEs, including platinum, incline to complex formation, hydrolysis, hydration, redox transformations, polymerization, etc. and hence exist in aqueous solutions as many chemical forms. Labile metal-ligand forms can furthermore undergo changes in the partitioning of a PGE among its species. This would compromise the determination of a given speciation, unless measurable interconversion does not occur during the course of a CE separation which typically takes less than 10 min. For instance, several groups [4,7,47,48] demonstrated an excellent resolution of Pt(II)–Pt(IV) chlorides mixtures (see also Fig. 2). However, only the

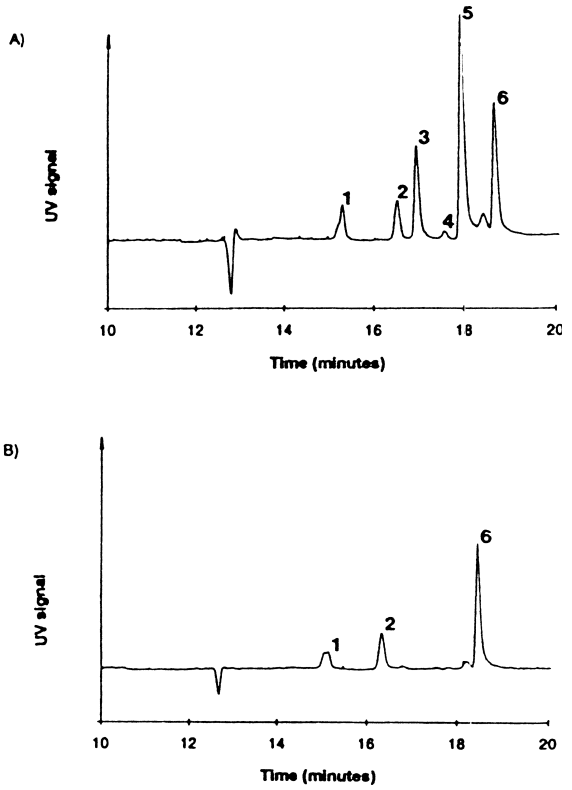


Fig. 6. Electropherograms of a leaching solution (A) before and (B) after 80 min in contact with activated charcoal. Conditions: capillary, fused-silica, 80 cm×50 μm I.D.; electrolyte, 20 m*M* Na_2HPO_4, 100 m*M* NaCl, 3 m*M* NaCN, 1.2 m*M* TBAB, 40 μ*M* TTAB (pH 11.0); sample introduction, vacuum; voltage, −15 kV; direct UV detection at 208 nm. Peaks: (1) $Cu(CN)_3^{2-}$; (2) NO_3^- (a depletion product of CN^-); (3) $Ni(CN)_4^{2-}$; (4) $Pd(CN)_6^{2-}$ (0.99 mg l^{-1}); (5) $Pt(CN)_4^{2-}$ (47 mg l^{-1}); (6) $Fe(CN)_6^{4-}$. (Reprinted with permission from Ref. [15]).

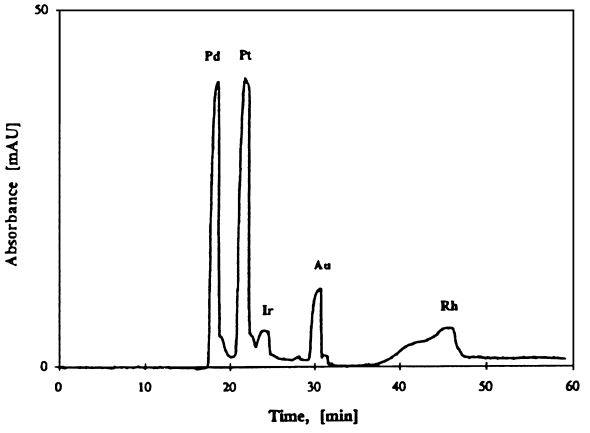


Fig. 7. Determination of precious metals in a multi-element standard 26H SM-40 (100 mg l^{-1} each precious metal). Sample pretreatment, see Table 1. Conditions: capillary, fused-silica, 75 μm×75 cm I.D.; electrolyte, 50 m*M* CH_3COONa, 30 m*M* $HClO_4$, 50 m*M* NaBr (pH 3.0); sample introduction, vacuum; voltage, −10 kV; direct UV detection at 235 nm. (Reprinted with permission from Ref. [16]).

platinum(II) complex injected from freshly prepared solution gave essentially a single peak that conforms a slow species transfer compared to analysis time. For platinum(IV), more easily affected by hydrolysis, a major peak as well as a much smaller slowly migrating peak of a complex in which one of the chloride ligands is replaced by water or hydroxyl ion, were recorded [4,7,47,48]. The increased acidity and higher chloride concentration could slow down the rate of hydrolysis of the Pt(IV) chloride. Indeed, no peak of the hydrolysis product was observed using an electrolyte solution containing 0.1 *M* HCl and 0.4 *M* NaCl [9] but this improvement was at the expense of a longer migration time, poorer peak shape and a less stable baseline. On the other hand, time-dependent species transformations for platinum (and other PGEs) can be monitored efficiently applying the CE method [4,7,9,43,46,49] (for more detail see Section 5). In the study recorded in Ref. [46], the differentiation of $[PtCl_6]^{2-}$ from the Pt–methionine complex as well as from other Pt species generated during aging of the solution was demonstrated. The peaks were identified by standard addition and by characteristic UV spectra recorded on-line.

The development of a CE methodology that works well for direct analysis of trace element species, including platinum [46,48,50], has been the impetus of considerable research efforts by Michalke and Schramel. The authors achieved the promising sensitivity of a laboratory on-line CE–ICP-MS system by employing a two-step procedure, which means that after the separation the high voltage was switched off and the separated species were moved to the detector by applying pressure to the capillary inlet. In such a way, a ca. 10-fold improvement in detection of $[PtCl_6]^{2-}$ over a conventional mode of operation (without the division of separation from detection) was gained [51]. A further decrease of the concentration detection limit, down to 1 μg l^{-1}, was possible after large-volume sample stacking. How-

ever, even with this threshold CE lags about 40 times behind HPLC–ICP-MS [43].

4.2. Iridium and rhodium complexed forms

Much less CE data has been used to assess the distribution of complexed species of iridium [7,52], rhodium [7,53] and osmium [4,7], mostly in hydrochloric acid medium. The work by Thornton and Fritz [7] indicated the presence of four species in iridium(IV) solutions, two of them being ascribed to Ir(III) compounds. A later detailed investigation [52] revealed a much more complicated speciation pattern for Ir(IV) chloro complexes in acidic media: at least seven species appeared in the electropherograms recorded for 0.001–1 *M* HCl solutions of the Ir(IV)–hexachloro complex. Such a diverse array of forms was proved to involve the formation of aqua-chloro, hydroxo-chloro and probably dimeric complexes, accompanied by a partial reduction of Ir(IV) to Ir(III). However, upon reaching the equilibrium the species present in the solution were mostly $[IrCl_6]^{2-}$, $[Ir(H_2O)Cl_5]^{-}$ and $[Ir(H_2O)Cl_5]^{2-}$, the result which is more complementary to data of the above paper [7].

The partitioning of Rh(III) among its species in different acidic environments was investigated by Aleksenko et al. [53]. The use of simple acidic electrolytes enabled separation of rhodium complexes similar in structure but varying in the charge state without a notable change in speciation and, in combination with diode-array detection, identification of the most abundant forms. Particularly, four differently charged complexes, existing in 0.1 *M* HCl, were separated and identified as depicted in Fig. 8. As could be expected, a less polymorphic speciation—three peaks due to $[RhCl_6]^{3-}$ and its hydrolysis products—was established in 11 *M* HCl (cf. with data of Ref. [7]). According to CE assay, the aquatic cationic species were predominant in $HClO_4$ and HNO_3 solutions, whereas only negatively charged forms of Rh(III) occurred in sulfuric acid. The speciation information gained in that and a subsequent report [54] allowed further insight into the mechanism of catalytic kinetic reactions utilized for highly sensitive determination of Rh(III).

The only contributions have been concerned with

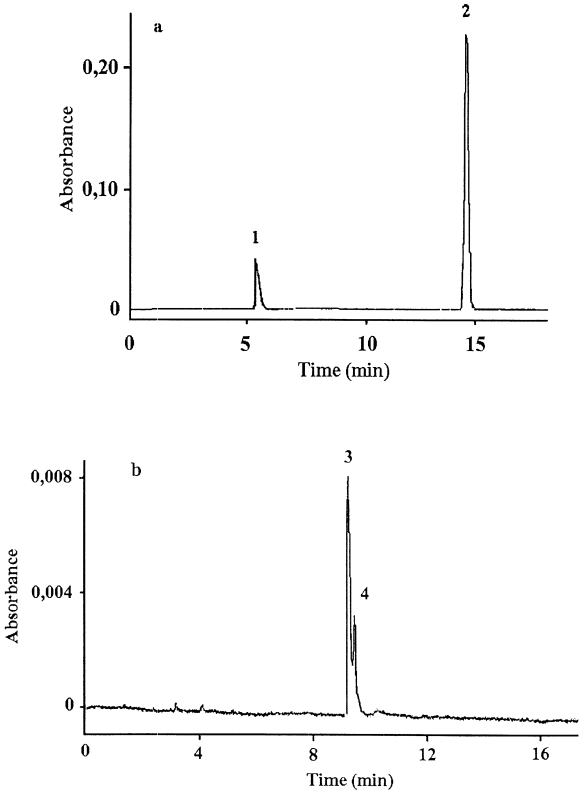


Fig. 8. Speciation of rhodium(III) chloro complexes in 0.1 *M* HCl. Conditions: capillary, fused-silica, 50 μm×64.5 cm I.D.; electrolyte, 10 m*M* KCl–HCl (pH 4.0); sample introduction, pressure (10 s at 5 mbar); voltage, (a) 30 kV, (b) −30 kV; direct UV detection at 200 nm. Peaks: (1) $[Rh(H_2O)_4Cl_2]^{+}$; (2) $[Rh(H_2O)_3Cl_3]$; (3) $[Rh(H_2O)_2Cl_4]^{-}$; (4) $[Rh(H_2O)_2(OH)Cl_3]^{-}$. (Reprinted with permission from Ref. [53]).

species of other PGEs, palladium [4] and ruthenium [7].

4.3. Platinum speciation in environmental samples

A series of studies deal with characterizing and measuring Pt species associated with soil by taking advantage of an on-line interfacing of CE with ICP-MS for element-specific detection [43,48–50,55–57]. A prerequisite for such speciation measurements is that the species are not impaired (or new species generated) during the separation and detection steps. Therefore, species stability was systematically checked out. Also, to exclude undesired transformations of chloroplatinates in aqueous extracts from

Pt-treated soil, which may occur before analysis, the extracts were frozen and then analyzed immediately after thawing. Specific CE patterns corresponding to the formation of various platinum forms due to rapid (within days) interaction of $[PtCl_6]^{2-}$, $[PtCl_4]^{2-}$ or metallic Pt with the soil were evident on examining the soil extracts, all the species being apparently recognized as bioavailable Pt forms. Aqueous extracts of a clay-like humic soil, treated with a Pt-contaminated highway tunnel dust, exhibited a different pattern of soil-generated species compared with artificial Pt species. Fig. 9 demonstrates such a pattern, comprising a variety of chloroplatinates and soluble platinum–humic acid associates, and additionally confirms the suitability of the CE–ICP-MS coupling to monitoring the dynamic changes in the Pt species–soil systems.

Weber and his co-authors [44,45] applied CE as a technique complementary to HPLC for the characterization of low-molecular-mass fractions (<10 000) of platinum species in grass, another important environmental matrix playing a role in mobilization and formation of bioavailable Pt forms and their translocation into the food chain. Only one main peak was obtained for the platinum species in fractions isolated by ultrafiltration, gel-chromatography and preparative isotachophoresis, and in total, separation and detection (UV and amperometry) data allowed the authors to attribute the platinum binding ligands in a rather plausible way to partly oxidized sugars.

Lustig et al. [56] designed and carried out special experiments to demonstrate that no contaminating artifacts due to the platinum release from the electrodes during electrophoresis take place. This important study underlines the relevance of the data of the above described speciation trials and proves that CE equipment can be used without modification (such as, e.g. replacing the Pt electrodes with ultrapure gold electrodes) whenever investigating platinum species.

5. Studies of platinum anti-tumor drugs

Since the discovery of cisplatin as a cancerostatic compound (see Table 2 for structural formula here and hereafter), many platinum complexes have been evaluated in an attempt to improve the anti-tumor activity and to reduce the severe side-effects such as neurotoxicity and nephrotoxicity [58]. An efficient separation technique, enabling investigations of platinum cytotoxic agents in aqueous solutions and biological media, was of special concern for pharma-

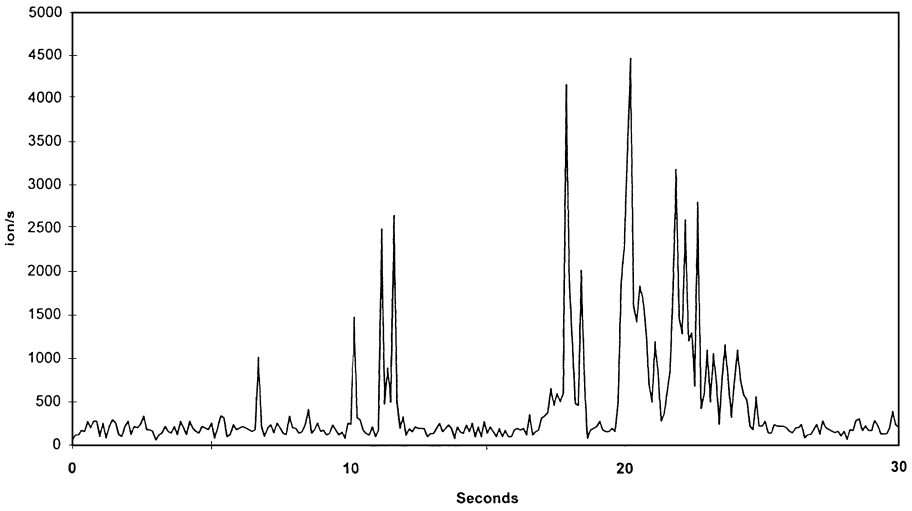


Fig. 9. Electropherogram of an aqueous extract from the soil after 7 days of interaction with Pt-containing tunnel dust. Conditions: capillary, fused-silica, 150 cm×50 μm I.D.; separation electrolyte, 20 m*M* NaH_2PO_4–Na_2HPO_4 (pH 5.6); stacking electrolyte, 100 m*M* phosphate buffer (pH 2.3); sample (and stacking electrolyte) introduction, pressure (15 s at 8 bar); voltage, 12 kV; ICP-MS detection, 195 *m*/*z*. (Reprinted with permission from Ref. [56]).

Table 2
Platinum metallo-complexes with antitumorogenic properties, their major metabolites and bioadducts with mononucleotides studied by CE[a]

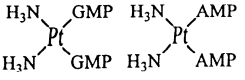
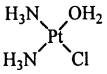
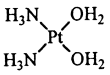
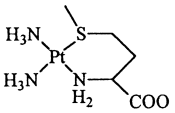
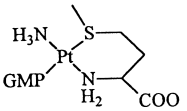
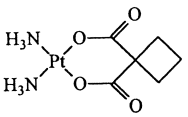
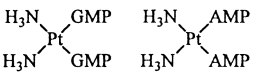
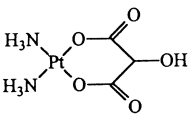
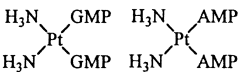
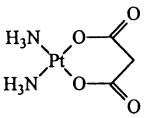
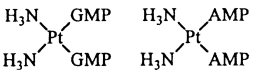
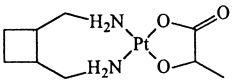
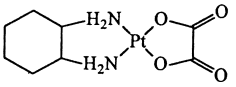
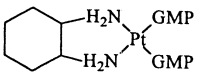
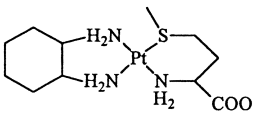
Name	Structure	Nucleotide adducts	Ref.
Cisplatin [*cis*-diamminedichloro platinum(II)]			[50,62, 65,67, 69,71]
cis-Diammineaquachloro platinum(II)			
cis-Diamminediaqua platinum			
cis-Diammine(methionine) platinum(II)			
Carboplatin [*cis*-diammine-1,1-cyclobutanedicarboxylato platinum(II)]			[62,68, 69]
cis-Diammine(2-hydroxymalonato) platinum(II)			[68]
cis-Diammine(malonato) platinum(II)			[68]
Lobaplatin [1,2-di(amminomethyl) cyclobutaneplatinum(II) lactate]		–[b]	[59]
Oxaliplatin [*trans*-R,R-diammino cyclohexane(oxalato) platinum(II)]			[69,72]
trans-R,R-Diammino cyclohexane(methionine) platinum(II)			

Table 2. Continued

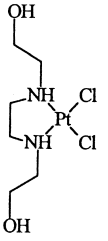
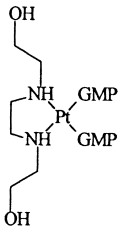
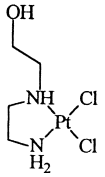
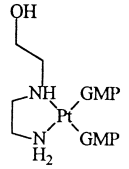
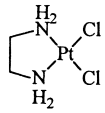
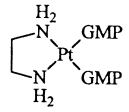
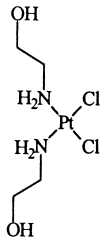
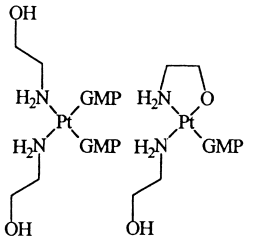
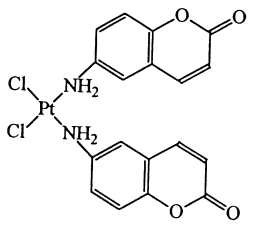
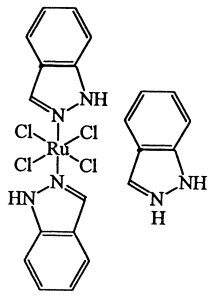
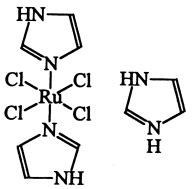
Name	Structure	Nucleotide adducts	Ref.
cis-Dichloro[*N*,*N*′-bis(2-hydroxyethyl)ethane-1,2-diamine]platinum(II)			[67]
cis-Dichloro[*N*′-(2-hydroxyethyl)ethane-1,2-diamine]platinum(II)			[67]
cis-Dichloro(ethane-1,2-diamine)platinum(II)			[67]
cis-Dichlorobis(2-hydroxyethylamine) platinum(II)			[67,73]
cis-Dichlorodi(6-aminocoumarin) platinum(II)		–[b]	[61]
Indazolium *trans*-tetrachlorobis(indazole) ruthenate(III)		–[c]	[70]

Table 2. Continued

Name	Structure	Nucleotide adducts	Ref.
Imidazolium *trans*-tetrachlorobis(imidazole) ruthenate(III)		–[c]	[70]

[a] Charges are omitted for simplicity.
[b] Not studied.
[c] Not identified.

ceutical and biomedical analyses. Yet in 1994 Vogt and Werner [59] drew attention to the spectacular abilities of CE to estimate the speciation of heavy metal complexes in the fields of medicine, pharmacology and ecology. Particularly, diastereoisomers of lobaplatin, a third-generation anticancer platinum complex, spiked into serum were separated using the micelle-forming SDS, i.e. under MEKC conditions. Such a separation is a necessary step to control the efficiency of dosages given to patients. While characterizing CE by high resolving power and extensive possibilities of varying the separation conditions, the authors first came into conflict with too high detection limits for real plasma analyses (as a consequence of a relatively low UV absorption of platinum compounds). Since then there is a growing comprehension that CE requires substantial gains in sensitivity with regard to potential clinical application and hence much more research devoted to establishing suitable detection methodology.

To understand the mode of action of established and new platinum-based tumor-inhibiting complexes at the molecular level, particular interest is being focused on their chemical transformations that occur under physiological conditions and on interactions of intact drugs and their metabolites with biologically relevant molecules (see Table 2). Most important reaction pathways are assumed to include hydrolysis, directly affecting the drug stability, and binding toward nucleotides and DNA fragments, the main target of platinum chemotherapeutics, as well as toward sulfur-containing biomolecules exhibiting a specific affinity to platinum.

5.1. Stability and kinetic measurements

Besides the reasons mentioned above, novel platinum cancer chemotherapeutics are synthesized in order to increase the drug's solubility and stability as well as to modify time-dependent binding properties with respect to nucleotides (see the following subsection). In aqueous solution, mainly positively charged platinum species exist in addition to the neutral complexes. For separation and determination of these species, HPLC with gradients of organic modifier concentration and/or ionic strength can be employed. However, the problem of resolving simultaneously complex mixtures of neutral, cationic and anionic platinum forms and interaction of the analytes with the eluent and possibly sorbent still remains. Choosing CE instead of HPLC represents a valuable alternative owing to the technique's well-known merits of operation in aqueous medium and separation of differently charged species (see e.g. Fig. 8).

Promising results using MEKC for separating various platinum drugs and their hydrolytic products and for kinetic measurements of complex stability in aqueous solutions were presented in a study by Wenclawiak and Wollmann [60]. The SDS concentration, pH, applied voltage and sample introduction conditions were optimized to attain a single-run separation of cisplatin, [*cis*-diammineaquachloroplatinum]$^{+}$ and [*cis*-diamminediaquaplatinum]$^{2+}$. This allowed for assessment of the stability of cisplatin in water and sodium chloride solutions of 100 (the concentration in blood) and 4 m*M* (the cytoplasm

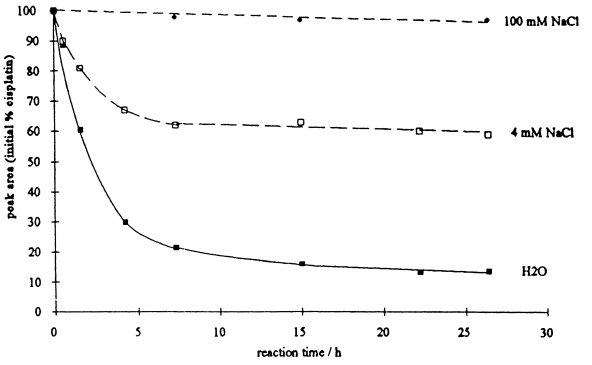


Fig. 10. Kinetic curves for cisplatin hydrolysis at 37 °C in aqueous solutions with different concentrations of sodium chloride. (Reprinted with permission from Ref. [60]).

concentration) by following the relative decrease of the drug peak area. As can be seen in Fig. 10, cisplatin decline depends substantially on chloride concentration; higher concentrations prevent hydrolysis.

The half-live times for the hydrolytic decomposition of *trans*-$[RuCl_4(im)_2]$ (im=imidazole) and *trans*-$[RuCl_4(ind)_2]$ (ind=indazole), which are the only non-platinum tumor inhibiting complexes studied by CE, were determined by Küng et al. [61]. Whereas the hydrolysis rate of the imidazole complex was found to be virtually pH-independent, the indazole complex hydrolyzed much faster at higher pH. The latter finding deserves a special consideration in view of the lower pH in solid tumors.

5.2. Interactions with biomolecules

The anti-tumor activity of platinum complexes is assumed to be due to the platination of the bases of DNA. Consequently, studies of adducts formed by different nucleotides became the focus of an ever growing interest. Analysis of nucleotides and DNA fragments by CE or capillary polymer sieving electrophoresis has been performed routinely. A first attempt to investigate platinated DNA by means of CE was undertaken by Sharma et al. [62], who applied a laser-induced fluorescence detector for assaying DNA damage after the exposition to *cis*- and carboplatin. Earlier contributions dealing with the quantification of platinated DNA relied on isolation of the exposed DNA followed by either determining the total Pt content by AAS or measurements of the total adduct level using immunological techniques. However, the sensitivity of the CE method proposed enable to detect as low as one adduct within 10^4 nucleotides has not been reached regardless of the approach employed. On the other hand, since the analytes under investigation lack the native fluorescence, samples have to be labeled with a fluorescence marker prior to CE analysis. Troujman and Chottard [63] compared the performance of HPLC and CE for the separation of the platinum adducts formed upon reaction of a double-stranded oligonucleotide, namely an 18-mer, and a single-stranded octamer with the two complexes, *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ regarded as the main hydrolysis product of cisplatin, and *cis*-$[Pt(NH_3)_3(H_2O)]^{2+}$. In general, the CE separations afforded higher efficiency and time saving. Moreover, the longer the oligonucleotide the better the resolution provided by CE became. Recently, preparative CE in combination with off-line graphite furnace AAS was applied to the analysis of DNA complexes formed by *cis*-dichlorodi(6-aminocoumarin)platinum(II) [64]. The platinated DNA was hydrolyzed by an enzymatic reaction and analyzed subsequently by CE. The oligonucleotide fractions were then collected (without interruption of the current) and the Pt content quantified down to 0.78 ng per a platinated DNA species. Using this combined technique the cytotoxic activity of the Pt–coumarin complex through interaction with DNA was evidenced.

Interactions of cisplatin, carboplatin and analogues with nucleoside monophosphates, di- and trinucleotides were systematically explored by Keppler and co-workers by applying CE coupled to a diode-array detector [65–69]. The upper trace of Fig. 11 shows an exemplary separation of all four common nucleotides and their major adducts originated from the incubation with *cis*-diammine(2-hydroxymalonato)platinum(II). Adduct formation resulted in a significant shift of λ_{max} to a lower energy region for modified nucleotides compared to free nucleotides. Therefore, identification of individual platinum-nucleotide adducts can be performed on the basis of both the characteristic UV spectra and differences in the electrophoretic behavior.

The formation of covalent platinum–nitrogen cross-links with DNA nucleotides occurs through the

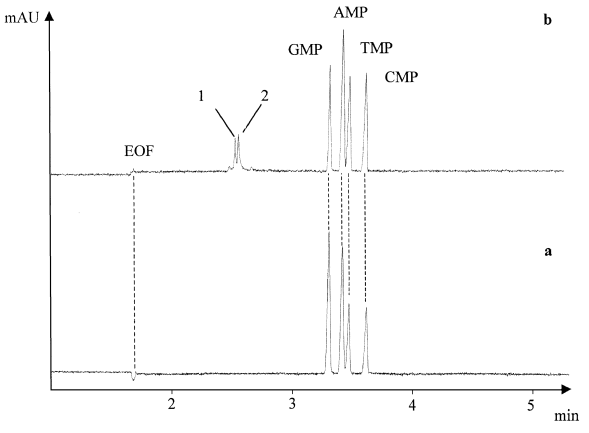


Fig. 11. Competitive interaction study of nucleoside monophosphates with *cis*-diammmine(2-hydroxymalonato)platinum(II). (a) Control sample (equimolar amounts of components); (b) after incubation with the platinum complex. Conditions: capillary, fused-silica, 50 μm×50 cm I.D.; electrolyte, 20 m*M* NaH_2PO_4–Na_2HPO_4, pH 7.0; sample introduction, pressure (10 mbar for 15 s); voltage, 30 kV; direct UV detection at 254 nm. Abbreviations: AMP, adenosine 5′-monophosphate; CMP, cytidine 5′-monophosphate; GMP, guanosine 5′-monophosphate; and TMP, thymidine 5′-monophosphate. Peaks: (1) *cis*-$[Pt(NH_3)_2(GMP\text{-}N7)_2]^{2-}$; (2) *cis*-$[Pt(NH_3)_2(AMP\text{-}N7)_2]^{2-}$. (Reprinted with permission from Ref. [68]).

position N7 of guanine as the most preferred binding site (and to a lower extent at N7 of adenine). Therefore, the majority of CE trials involved 5′-guanosine monophosphate (5′-GMP) as a model substance. The kinetics of binding properties of 5′-GMP toward cisplatin under simulated physiological conditions, with a special concern of the chloride concentration in the inter- and intracellular space, was examined by the same group [66]. The formation of adducts was proved to be affected significantly by the presence of chloride ions. Furthermore, the influence of the sulfur-containing α-aminoacids, L-methionine and L-cysteine, on the rate of adduct formation was explored in consideration of the high affinity of sulfur to platinum [66,69]. By preferential coordination of such ligands an inactivation of platinum-containing chemotherapeutics may take place. Both electrophoresis and incubation of the samples were carried out by use of a phosphate buffer with a 4 m*M* chloride concentration and a pH of 7.4 which simulates intracellular conditions. The presence of L-methionine and L-cysteine increased the half-time of 5′-GMP-coordination and in both cases free 5′-GMP could still be detected at the end of the observation period. Investigations on the interaction between ruthenium(III) complexes (see Table 2) and nucleotides revealed that the kind as well as the rate of adduct formation depends on the pH of the incubation buffer system [70]. However, the structure of the adducts could not be identified.

With UV detection, in fact only limited structural information about platinum–DNA adducts is available. The CE's miniaturized format hinders on the other hand the recovery of sufficient amount of material required for product identification by means of spectroscopic or MS techniques. The problem of exact peak characterization can be solved most straightforwardly with the help of MS on-line coupled via an electrospray ionization (ESI) interface. ESI is a soft ionization process capable of bringing quite large molecules of interest into the molecular mass range of conventional mass spectrometers thus acquiring important knowledge on molecular mass, state of charge, isotopic pattern and molecular structure. Warnke et al. [71] reported recently the first application of a CE–ESI-MS system in characterizing platinated DNA nucleotides. After incubation of mononucleotides with cisplatin, a series of monochloro, monoaqua and bifunctional adduct species were separated and identified. Moreover, the method's feasibility to monitor the kinetics of adduct interconversion was demonstrated. CE–ESI-MS has been further exploited to elucidate the binding behavior of oxaliplatin to 5′-GMP in the presence of L-methionine [72] (Fig. 12) and to analyze directly adducts generated from the reaction of *cis*-dichlorobis(2-hydroxyethylamine)platinum(II) with 5′-GMP [73]. As seen in Fig. 12, selected ion monitoring provides a selective means for monitoring interconversion of the parent Pt complex into its methionine derivative; insets show the mass spectra of initial and reaction products.

A supplementary approach in the characterization of platinum–bioligand complexes takes advantages of applying selective spectroscopic methods (e.g. NMR) or molecular-mass-specific MS modes, such as ESI-MS and matrix-assisted laser-desorption-ionization (MALDI) MS. In this regard, the preliminary report of Wagner et al. [74] should be mentioned. The authors used successfully two dimensional NMR

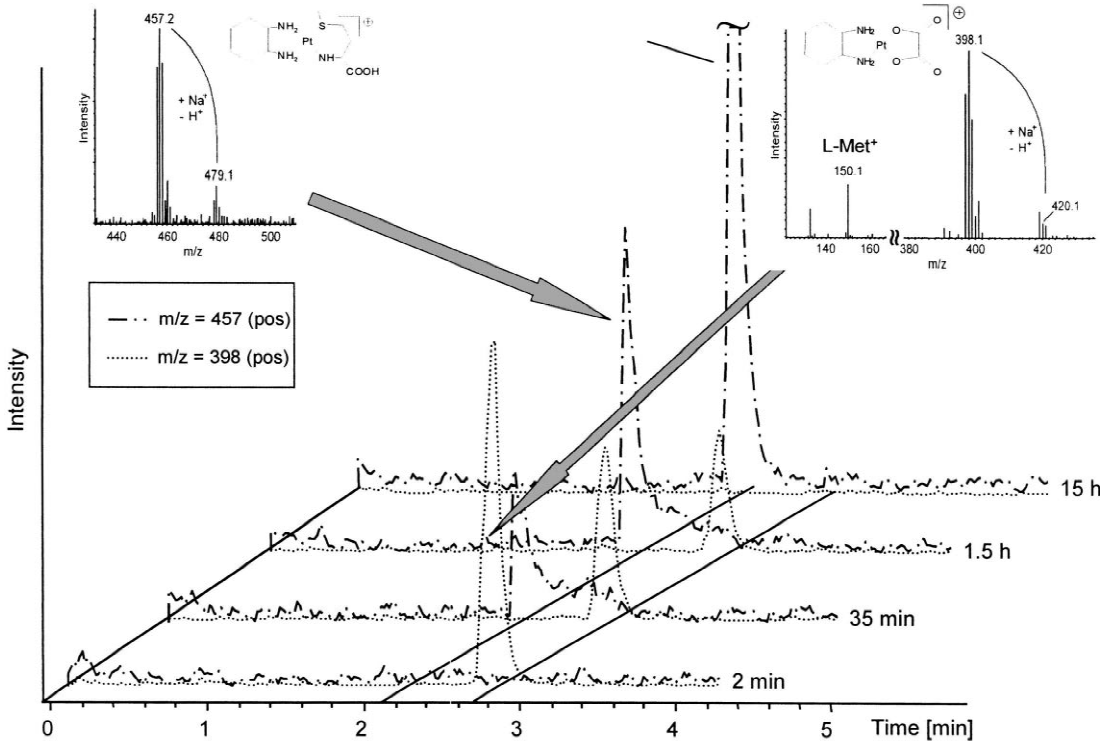


Fig. 12. Single ion electropherograms of an oxaliplatin-L-methionine mixture recorded after the specified reaction time. Conditions: capillary, fused-silica, 60 cm×50 μm I.D.; electrolyte, 30 m*M* ammonium carbonate (pH 7.4); sample introduction, pressure (50 mbar for 10 s); voltage, 25 kV; ESI-MS detection via a sheath–liquid interface [methanol–water–concentrated acetic acid (80:19:1, v/v/v), 4 μl min^{-1}]. (Reprinted with permission from Ref. [72]).

spectroscopy to distinguish the cisplatin-dinucleotide reaction products. In combination with MEKC separation three distinct individual isomers of the Pt adduct were identified. The applicability of MALDI-MS [67] and ESI-MS [69] as techniques complementary to CE for more precise identification of adducts formed by various platinum complexes was also demonstrated. Specific molecular mass information obtained facilitated greatly the assignment of peaks in UV electropherograms.

6. Conclusions

Element speciation analysis and investigations on metal interactions with bio(macro)molecules are without doubt two major areas where CE has gained the status of a competitive technique over a period of years. Moreover, in the authors' opinion, the method's prospects can be largely related to application areas in which CE has a number of decided advantages over HPLC. These are the circumstances that underscored the need for an overview focussed particularly on the methodology and practices of CE on discriminating the speciation pattern of PGEs and recognizing possible biotransformations of tumor-inhibiting PGE complexes. To date, however, relatively little use has been made of CE in the determination of the PGE speciation in real samples. Likewise, still challenging remains the development of a CE methodology for studying the metabolism and mode of action of platinum anticancer drugs under physiological conditions and for determining their adducts in cell material (e.g. after enzymatic degradation of platinum-modified cellular DNA). The very low environmental and physiological concentrations of PGEs on the one hand and limitations in detection sensitivity of CE method and non-selec-

tive character of commonly used detectors on the other are the primary reasons. Improvements in performance of the CE–ICP-MS system, following a custom-designed interface to be put into effect, and more judicious implementation of ESI-MS for structurally informative CE assays represent therefore important facets on which further work should be increasingly centered. Other anticipated directions along which advancements in CE applied to both areas will likely take place encompass sample preparation procedures ensuring PGE species stability and avoiding matrix effects, assessing and preserving the possible changes in initial species distribution during electrophoresis, performing reliable identification and quantification of species from complex matrices, elaboration of the detailed guidelines on CE method validation and system suitability, etc. [46,75]. The respective advanced approaches and solutions desirable for assaying every element species are prominently important for PGEs often showing fast species transformations.

At present, accurate determinations of total concentrations of each of the PGEs by CE have only been possible in industrial samples, technical materials and pharmaceuticals. When the expected advances in detection methodology come true, one may envisage that such measurements will be extended to include environmental and clinical matrices. However, it is rather speculative to expect that the application potential of CE, even coupled to high-resolution ICP-MS, in this field might become comparable with that of instrumental methods, such as adsorptive stripping voltammetry or electrochemical atomization laser-excited fluorescence spectrometry, which already enjoy excellent sensitivity, and the equipment currently available in many research laboratories.

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