Detection of Platinum Species in Plant Material By Preparative Isotachophoresis Using the Model 491 Prep Cell

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Abstract

An isotachophoretic method is presented for the separation of platinum containing proteins from plant material in the Model 491 Prep Cell. Platinum-containing species in extracts of grass cultivations were first separated by gel chromatography, then by isotachophoresis. This combined separation procedure leads to an excellent separation unobtainable by other means. Isotachophoresis provides a useful preparative method that expands the separation capabilities of the Model 491 Prep Cell.

Introduction

Heavy metals are of great importance in environmental research. Besides the determination of baseline levels of trace elements in biological and environmental materials, the investigation of the bio-utilization of heavy metals is of increasing interest. Knowledge of the binding state and binding partners of an element in biological and physiological systems supplies information about its bio-availability and consequently of its toxicological and nutritional relevance.

A separation method for analysis of metal-containing species must fulfill at least two main criteria. It should work on a preparative scale because of the expected low concentrations of metals in native biological material. The method should also not impair the binding between trace elements and the organic components to be purified; that is, it must function under physiological conditions.

Platinum emission from cars with catalytic cleaning of the exhaust gases represents a newly added factor of environmental pollution.[1] The emission of platinum as small particles (sub-µm range) increases the bio-availability of this element[2,3] and has created interest in biological studies of platinum. For the separation and characterization of platinum species in grass cultivations, preparative isotachophoresis with the Model 491 Prep Cell leads to excellent separations. Sharply isolated platinum-containing fractions were obtained, which were further characterized biochemically.

Methods

The combined procedure for the separation, detection, and characterization of platinum-containing species in plant materials is described in references 4 and 5.

Briefly, extracts of untreated and platinum-treated grass cultivations were separated by combined use of two different preparative methods with different separation principles: gel permeation chromatography followed by isotachophoresis in the Model 491 Prep Cell. This combined procedure leads to an excellent recovery of purified platinum binding proteins. The detection of the platinum species was carried out sequentially by absorptive voltammetric platinum determination in individual fractions. Native electrophoresis (PAGE) was used for further characterization of selected species.

Isotachophoresis

Following chromatography[4,5], a second preparative separation step of isotachophoresis gave best results. In isotachophoresis, the upper buffer is called the terminating electrolyte and the lower buffer is the leading electrolyte. The non-restrictive polyacrylamide gel is polymerized containing the leading electrolyte. During electrophoresis, charged molecules electrophorese through the gel with the same velocity and in the order of their net mobilities. The net mobilities of the sample ions must be between the mobilities of the leading electrolyte and the terminating electrolyte. When the system has reached equilibrum, each zone travels in immediate contact with its neighbors and the velocity of these zones is the same as that of the leading electrolyte. The electrochemical process is similar to the more familiar stacking process, but more extended geometrically. Each zone contains only the sample component and the counter ion. In isotachophoresis, there is an efficient counteraction of diffusion as a consequence of a zone sharpening phenomenon. Sample zones maintain sharp boundaries resulting in high resolution and highly concentrated zones.

In order to resolve the isotachophoretically-separated zones it is necessary to add spacer ions, consisting of carrier ampholytes, to spatially separate sample zones.

Running Conditions

The conditions of the isotachophoretic separation technique with the Model 491 Prep Cell were as follows.

The 28 mm ID gel tube was used.

1. Separating gel (non-restrictive gel): A monomer concentration of 5% T with a crosslinker concentration of 5% C was used. A 100 ml amount of the leading electrolyte was mixed with 4.75 g of acrylamide and 0.25 g of N,N'-methylenebisacrylamide (Bis). After degassing this solution, 20 µl of TEMED and 200 µl of 10% (w/v) ammonium persulfate solution (100 mg ammonium persulfate + 1.00 ml water) were added to initiate chemical polymerization. The separating gel was cast in the tube assembly to a height of about 7 cm following the procedures detailed in the instruction manual. The monomer solution was overlayed with water-saturated 2-butanol. After polymerization, the butanol was removed and the gel surface was rinsed with water.

2. Stacking gel: For casting of the stacking gel, a monomer concentration of 2.5% T with a crosslinker concentration of 20% C was used. Fifty ml of leading electrolyte were mixed with 1.0 g acrylamide and 0.25 g Bis. After degassing, 30 µl of TEMED and 300 µl of 10% ammonium persulfate solution were added. The stacking gel solution was cast on the separating gel to a height of 1 cm. The stacking gel was overlayed with water-saturated 2-butanol. After polymerization the butanol was removed and the gel surface was rinsed with water. The gel was used for running after about 15 h in order to obtain complete polymerization.

The leading electrolyte (gel and lower anode buffer chamber) was 0.05 M HCl, pH 8.0 (the pH was adjusted by Tris); the terminating electrolyte (upper cathode buffer chamber) was 0.153 M 6-aminocaproic acid, pH 8.9 (Tris); the elution buffer was 0.02 M ammonium acetate, pH 8.0 (Tris).

The samples analyzed were selected fractions from gel chromatography that had been concentrated by freeze-drying. Carrier ampholytes were added to a sample solution of less than 3.0 mg (0.500 ml sample volume, pH 8.0, + 0.1 ml carrier ampholyte, pH 5 – 7, + 0.1 ml carrier ampholyte, pH 6 – 8, + 0.1 ml carrier ampholyte, pH 7 – 9). Bromophenol blue was used as a marker. The sample was carefully loaded on the surface of the stacking gel.

The operating conditions were:

Initial voltage	400 V constant
Initial current	6 - 12 mA
Final voltage	400 - 250 V
Final current	12 mA constant

The whole electrophoretic procedure, including polymerization of the gels, was carried out at 15 °C in a cold room.

Fraction Collection and Analysis

For elution, a peristaltic pump was set to a flow rate of 1ml/min. The absorption profiles of the eluates were continuously monitored at 280 nm. A total of 100 fractions were collected at 3 min intervals. The time for the complete isotachophoretic procedure was approximately 24 h.

For platinum detection, fractions were completely mineralized by an open, wet digestion step with nitric acid, perchloric acid, and sulfuric acid. Platinum was determined by our sensitive absorptive voltammetric method that has detection limits in the sub-pg range.[5]

Results

These investigations were performed with the aim of developing analytical methods for the separation and detection of platinum species in plant materials. It was shown that by an approach combining gel chromatography and isotachophoresis, platinum-containing species in grass samples could be separated with high resolution.[4,5] Figure 1 shows the UV profile and platinum distribution of the isotachophoresis step. Very sharp and concentrated platinum peaks were obtained. The

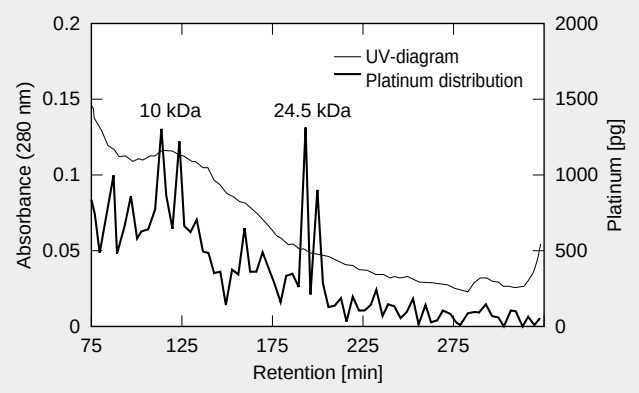

Figure 1A. UV diagram and platinum distribution of an isotachophoretic separation (UV-diagram absorbance at 280nm) of concentrated fractions (molecular mass range <45kDa) after gel chromatography.

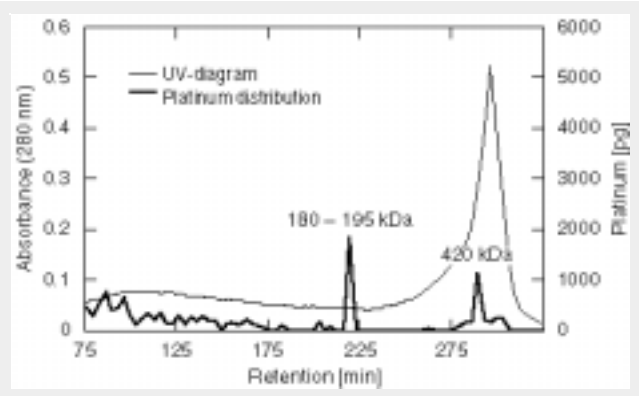

Figure 1B. UV-diagram and platinum distribution of an isotachophoretic separation (UV-absorbance at 280nm) of concentrated fractions (molecular mass range >45kDa) after gel chromatography.

platinum of the corresponding peaks eluated in only one or two fractions as a result of isotachophoresis.

The molecular mass of these platinum-containing fractions were determined by native horizontal flat-bed electrophoresis (PAGE). By using our sensitive determination method it was possible to detect platinum in separated and stained protein bands after PAGE, followed by digestion of the excised gel segments.[5]

No other fractionation technique was as effective as isotachophoresis in purifying the very low abundance platinum species from grasses.

References

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