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Research Article

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Development and validation of a micellar electrokinetic capillary chromatography method for determination of cisplatin in tumor tissue

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ABSTRACT

Cisplatin [cis-diamminedichloroplatinum (II)] is an effective anti-neoplastic agent that has been used to treat various types of solid tumor. However, it is known to cause several side effects, thereby cisplatin therapy is particularly restricted to severe nephrotoxicity. To monitor the efficacy and safety of drug treatment, it is important to determine the concentration(s) of the active species in target tissues. Capillary electrophoresis (CE) allows the separation of a wide range of compounds with a unique combination of high efficiency, short analysis time, low cost with little sample and background electrolyte (BGE) consumption, making it an economical and eco-friendly approach. To achieve these goals, Micellar electrokinetic capillary chromatography (MEKC) was found to be the most suitable capillary electrophoresis mode. Therefore the aim of this work is to develop and validate a CE method to determine cisplatin concentration in tumor tissue. The optimized BGE composed of a mixture of 65 mM NaH_2PO_4, 40 mM $Na_2B_4O_7$ and 90 mM SDS at pH 8.5. Elution of cisplatin was obtained within 12 min with good linearity (R^2 = 0.9997, 0.9991) for concentrations of 20 to 320 mg.L^{-1} and 25 to 300 mg.L^{-1} for cisplatin in water and matrix respectively. The LOD values were 5.7 and 7.2 mg.L^{-1} for cisplatin in water and tumor tissue (biological matrix), respectively and the LOQ values were 19 and 24 mg.L^{-1} for cisplatin in water and in tumor tissue (biological matrix), respectively.

Keywords: Cisplatin, tumor tissue, Micellar Electrokinetic Capillary Chromatography.

INTRODUCTION

Cisplatin [*cis*-diamminedichloro platinum (II)], **(Fig. 1)** is an effective anti-neoplastic agent that has been used to treat various types of solid tumors, such as testicular and ovarian cancers. It is also used for treating bladder, cervical, oesophageal, small cell lung, head and neck cancers.

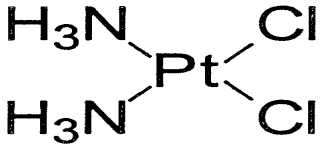

Figure 1. Chemical structure of Cisplatin [*cis*-diamminedichloroplatinum (II)]

Since cisplatin was discovered as an antineoplastic agent increasing the life-expectancy of cancer patients, it is the first-choice drug for chemotherapeutic agent of testicular and ovarian cancers [1–3]. However, its clinical dose-limiting nephrotoxicity and the occurrence of cellular resistance develop complications and limit drug's potential efficacy [4, 5]. Moreover, when overdosed, cisplatin might be genotoxic to mammalian cells and this harmful feature appears of a special interest due to the risk of inducing secondary malignancies. However, the modes of action and toxicity of cisplatin are not well understood. Therefore the concentrations of cisplatin after administration play an important role in estimating its side-effects and require accurate measurements. Likewise, a rapid and reliable analytical tool for determinations of intact cisplatin is a basic requirement in systematic cancer chemotherapy. The success in cisplatin-based chemotherapy, however, strongly depends on how careful the drug's dosages are monitored in order to reduce severe side-effects which include nausea, vomiting, kidney damage and deafness and to overcome cellular resistance. The damage produced in the affected organs is probably due to the association of Pt or the parent drug metabolites to important proteins of the impacted organ. The determination of cisplatin in biological fluids and tissues presents a particularly interesting challenge to the analytical chemist. Also, cisplatin is highly reactive in the body as its biodegradation products may have activity and toxicity behaviour different from that of the parent drug. It has been suggested that cisplatin and its hydrated species might be responsible for both its antitumor and toxic effects [6–8].

In order to evaluate the drug in clinical situations and to optimize therapeutic regimens, analytical methods capable of separating the drug and its individual biotransformation products and detecting these species at therapeutically relevant levels are required.

Bosch *et al*. [9] reviewed most important analytical procedures developed until 2008 for the determination of cisplatin by using selective methods.

Several methods have been applied for determination of cisplatin in various matrices including spectrophotometric [10], atomic absorption spectrophotometry (flame and non flammable) [11-14], inductively coupled plasma-atomic emission (ICP-AE) [15], electro analytical techniques [16, 17], neutron activation analysis [18], gas chromatography (GC) [19], liquid chromatography (LC) [20–25] and capillary electrophoresis (CE) [26]. LC is well suited for the separation of individual species of platinum-based drugs including assaying biological samples. However, this technique apparently suffers from interferences of complex biological samples. On the other hand, CE is free of such shortcomings and it has also a number of advantages over LC in terms of separation power, ease of use, sample consumption requirements, analysis time and accessibility of on-line preconcentration approaches. Therefore, this study considered of interest the development of a method in which cisplatin can be analyzed in that matrix by a CE method.

In this paper, a novel direct Micellar Electrokinetic Capillary Chromatography (MEKC) method was developed and validated for the direct determination of cisplatin in tumor tissue. This method is rapid and straightforward. MEKC has been used frequently due to its usefulness for the analysis of neutral and/or more hydrophobic substances. This method was used for the determination of cisplatin in mice tumor tissue samples.

EXPERIMENTAL SECTION

2.1. Reagents and samples

All chemicals used were of analytical grade. Cetyltrimethylammonium bromide (CTAB) was purchased from Merck (Darmstadt, Germany). Sodium tetraborate decahydrate, sodium dihydrogen phosphate and sodium dodecylsulfate (SDS) were purchased from Acros Organics (Geel, Belgium). Sodium hydroxide was purchased from Riedel-deHaën (Seelze, Germany). Cisplatin was purchased from Sigma (St. Louis, MO, USA).

All solutions were prepared by using ultrapure MilliQ-water (Millipore, Milford, MA, USA) and were filtered with a 0.2 µm membrane filter syringe from Whatman (Dassel, Germany).

The pH value of the buffers was measured and adjusted with the aid of a Metrohm 691 pH-meter (Herisau, Switzerland). Tetraborate buffers with pH values around 9.2 were prepared with sodium tetraborate, the pH was adjusted with solutions of sodium dihydrogen phosphate with molarity corrected for both Tetraborate and phosphate.

During method development, sample stock solutions of cisplatin was prepared at a concentration of 1.0 $g.L^{-1}$ in water and stored at 7 °C for at most one week.

2.2. Sample preparation

A stock solution of Cisplatin (1 g.L $^{-1}$) was prepared and used to prepare cisplatin standard solutions for calibration curves with concentrations ranging from 20–320 mg. L^{-1} in pure water and 25–300 mg. L^{-1} in the matrix.

To extract cisplatin, tumor tissues were lysed with solution (2 mM TFA: H_2O) 1:2. The homogenate (200 mg) of tissue was placed in a 1.5-mL Eppendorf tube and 400 µL H_2O was added and mixed for five minutes. Then samples spiked with 400 µL of Cisplatin (1.25 g.L $^{-1}$), to yield a final Cisplatin concentration of (500 mg.L^{-1}). Six solutions were prepared using Eppendorf tubes (0.5 mL) and were labeled from 1-6 containing 25, 50, 100, 150, 200, 300 µL solution and the volume made up with H_2O. Tubes were centrifuged for 30 min at a speed of 13147 *g* using a mini-Spin Plus (Eppendorf, Hamburg, Germany) and the supernatant was removed. Drying was performed by evaporating the solution at 45 ^{o}C for 50 min then reconstitute in 500 µL (1 mM TFA in H_2O). This was followed by centrifugation for 20 minutes at room temperature and 100 µL of the supernatant was transferred into a micro sample vial to be directly injected to CE system.

2.3. Instrumentation and operating conditions

The experiments were performed on a P/ACE MDQ instrument with diode array detector (DAD) and the data acquisition was done by means of 32 Karat^{TM} 4.0 software (both Beckman-Coulter, Fullerton, CA, USA). Uncoated fused silica capillaries of 75 µm id and 375 µm od were purchased from Polymicro Technologies (Phoenix, AZ, USA). The total length was 60 cm and effective length was 50 cm.

Unused fused silica capillaries were conditioned at 45 °C by rinsing with 1 M NaOH (10 min), 0.1 M NaOH (30 min), wait for 30 min and water (5 min), respectively. Daily at the beginning of analysis, the capillary was rinsed with 1 M NaOH (5 min), 0.1 M NaOH (3 min), water (1 min) and BGE (2 min); all the steps were performed at 25 °C and 138 kPa pressure. The inlet/outlet vials were replaced every 3 runs.

The separation voltage was optimized by plotting a curve of the generated current as the function of the applied voltage. The linear range (where Ohm's law is valid) was the working range and the maximum voltage in this range (15 kV) was adopted as the optimal separation voltage giving the best efficiency.

The capillary was rinsed between the runs for 1 min with 0.1 M NaOH, 1 min with water and 3 min with BGE at 138 kPa. Samples were hydrodynamically introduced at a pressure of 3.45 kPa for 5 s using a separation voltage of 15 kV at 25 °C.

RESULTS AND DISCUSSION

3.1 Method development and optimization

It is well known that the buffer pH and its ionic strength influence the electrophoretic mobility of the analytes during the electrophoretic process. The selection of the BGE constituents is therefore crucial in achieving good peak shape and efficiency. Furthermore, the peak resolution can be improved by using a surfactant as flow modifier such as SDS and CTAB.

According to the aim of the study, solutions of cisplatin were prepared and injected in order to define the best electrophoretic conditions to get the highest selectivity and sensitivity. Attention was paid to selectivity and separation of the analyte species from the matrix (tumor tissue) components without interferences.

The optimal separation conditions were found to be 65 mM NaH_2PO_4, 40mM NaB_4O_7 and 90 mM SDS at pH 8.5, a capillary temperature of 25 °C and separation voltage of 15 kV. Figures **2** and **3** Show a typical electropherograms of cisplatin in water and matrix (tumor tissue), respectively.

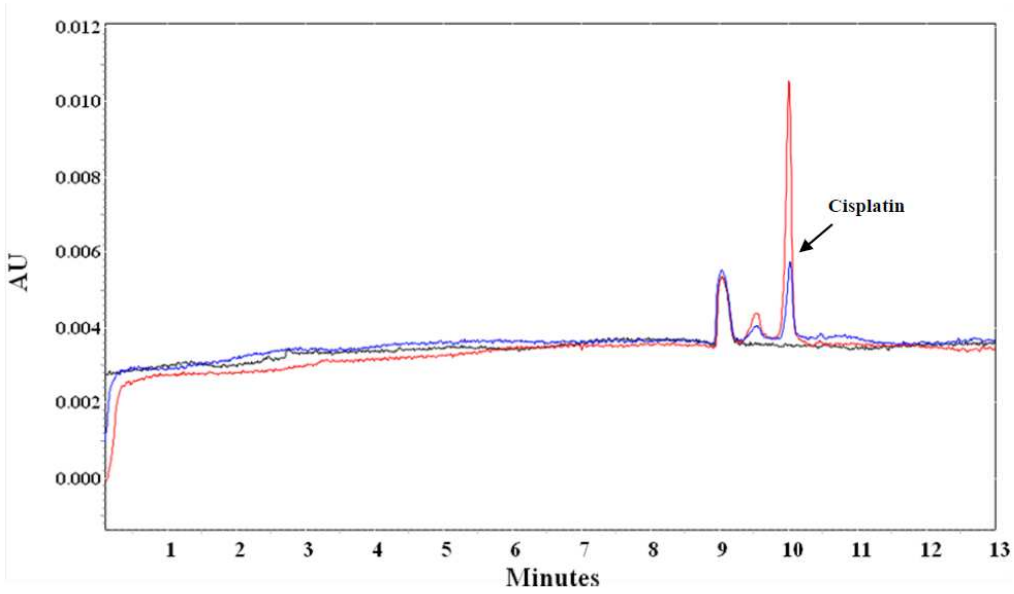

Figure 2. Electropherogram showing the separation of Cisplatin in water. CE conditions: voltage, 15 kV; BGE, 65 mM NaH_2PO_4, 40 mM $Na_2B_4O_7$ and 90 mM SDS at pH 8.5. A; (Black- bottom) blank (water), B; (Blue- middle) 40 mg.L^{-1} cisplatin and C; (Red- top) 120 mg.L^{-1} cisplatin in water.

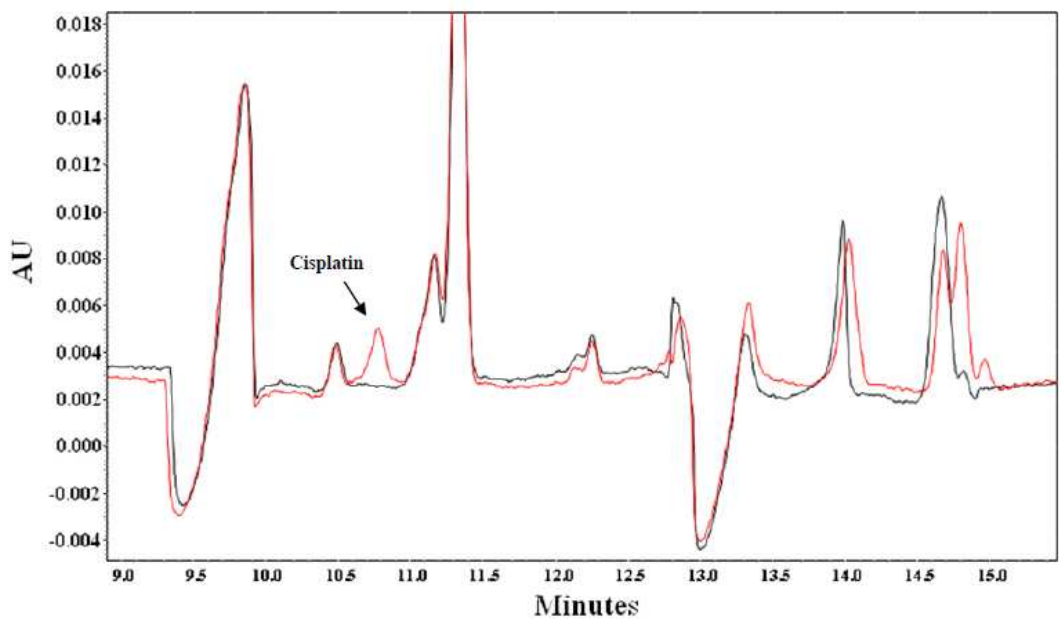

Figure 3. Electropherograms showing the separation of Cisplatin in water. CE conditions: same as specified for figure 2. A; (Black- bottom) blank matrix, B (Red- top); 50 mg.L^{-1} cisplatin spiked to tumor tissue (matrix).

3.2 Quantitative aspects

The precision, sensitivity and linearity of the method were evaluated as follows:

3.2.1 Sensitivity

For calculations, relative corrected peak areas were used. Limit of detection (LOD) and limit of quantification (LOQ) values are calculated as 3 and 10 times the signal-to-noise ratio (S/N). Good sensitivity was observed, the LOD values were 5.7 mg. L^{-1} in water and 7.2 mg.L^{-1} in tumor tissue. LOQ values were 19 mg. L^{-1} in water and 24 mg.L^{-1} in tumor tissue.

3.2.2 Accuracy- Recovery

The accuracy was determined by spiking the cisplatin to the water solution and homogenated tumor tissue (matrix) at two concentration levels as 50 mg.L^{-1} and 100 mg.L^{-1}. The recoveries were found to be 98.4 and 100.7 % for water solution samples and 95.6 and 97.8 % for the tumor tissue (matrix) samples.

3.2.3 Linearity

The optimized separation was obtained in less than 12 min with good linearity (R^2 = 0.9997 and 0.9991), with 6 concentration points in triplicates for the range from 20 to 320 $mg.L^{-1}$ and 25 to 300 $mg.L^{-1}$ for water solution and tumor tissue (matrix), respectively. The regression equations were obtained: y = 25.447x + 131.98 and y = 10.1302x + 1.1006, for water solution and matrix, respectively. Where y: relative corrected peak area, x: concentration ($mg.L^{-1}$).

3.2.4 Repeatability

The system repeatability was performed by using the same sample and BGE expressed as the relative standard deviation (% RSD) of the relative corrected peak area and migration time. Intraday precisions of the relative corrected peak area and migration time were 0.4 % and 0.2 % (n = 6), respectively and interday precisions of the relative corrected peak area and migration time over 3 days were 0.7 % and 0.3 % (n = 18), respectively. The means of migration time were 10.1 min (n = 6) for intraday precision and 10.1 min (n = 18) for interday precision.

3.3 Application of the optimized MEKC method for determination of cisplatin in real samples

A tumor tissue has been removed from mice treated with cisplatin, lysed as mentioned above in the **section 2.2** was investigated to determine the amount of cisplatin reached to the target tissue (**Fig.4)**.

Improvement in analysis time and simplicity was obtained but the sensitivity of the method may require further improvement.

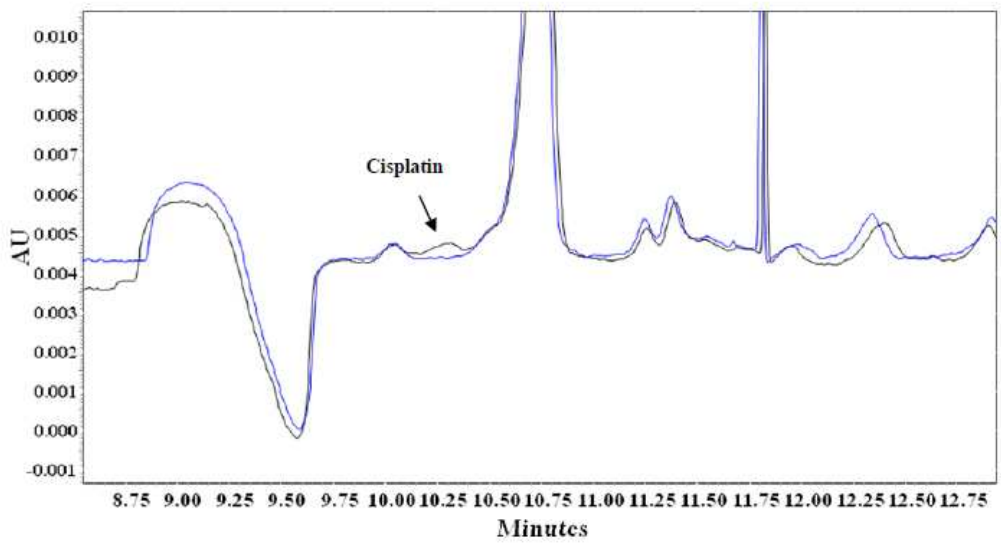

Figure 4. Electropherogram showing the separation of Cisplatin in water. CE conditions: same as specified for figure 2.A; (Blue- bottom) blank matrix, B; (Black-top) cisplatin in real tumor tissue sample (matrix).

CONCLUSION

The goal of this work was the development, optimization and validation of a selective, straightforward, simple and fast MEKC method for the determination of cisplatin in tumor tissues.

A MEKC method was developed, optimized and validated for linearity, inter- and intra-day precision, sensitivity and accuracy. Successful separation and good resolution between cisplatin and matrix peaks were achieved. The described method yielded an effective improvement in simplicity and analysis time (12 min). Based on all the above results, this direct MEKC method can be useful for the determination of cisplatin. It can make it a feasible and good alternative method.

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