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MICROWAVE-ASSISTED SYNTHESIS OF THE ANTICANCER DRUG CISPLATIN, *cis*-$[Pt(NH_3)_2Cl_2]$

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ABSTRACT

A microwave-assisted synthesis of cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, has been developed and optimized on both a 0.2 and 0.05 millimolar scale. The optimized synthetic procedure was modeled after the Lebedinskii-Golovnya method and is suitable for incorporating the radionuclide, ^{195m}Pt, into cisplatin for biological studies. Highest yields (47 %) and purity are obtained using a K_2PtCl_4:NH_4OAc:KCl molar ratio of 1:4:2 at a temperature of 100 ˚C. The entire synthesis and purification procedure requires approximately 80 min.

At a reaction temperature of 150˚C, the *trans* isomer is the exclusive product, suggesting that complexes of the general form, *trans*-$[Pt(RNH_2)_2Cl_2]$, can be synthesized directly from K_2PtCl_4 using $[RNH_3]OAc$ (R = alkyl or aryl moieties) via a microwave process.

Two novel separation procedures have been developed which efficiently remove the major impurity (1:1 Magnus-type salt) from the crude reaction product, yielding a product of purity comparable to that obtained by the Dhara method and suitable for biological studies. These procedures are applicable to both the micro and macro scale of synthesis.

The question of whether this microwave-assisted synthesis of cisplatin will be a preferred method for incorporating ^{195m}Pt into cisplatin is yet to be determined.

INTRODUCTION

Microwave technology is routinely used in organic syntheses because it offers several advantages over classical synthetic methods, such as dramatically shortening reaction times, increasing the range of possible products, and carrying out transformations that are not possible using conventional heat.[1,2] In contrast, this technology has not been investigated, to any appreciable extent, in the synthesis of transition metal complexes, in general, and in the synthesis of platinum complexes in particular. To date, Ru and Ir complexes are the most extensively studied inorganic systems using this technology.[3]

We have initiated a broad program to explore the use of microwave technology (heating) as an effective tool in synthesizing 2nd and 3rd row Group VIII metal complexes. We present here our first completed study: the optimized microwave-assisted synthesis of cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, the first FDA-approved Pt(II) drug whose potent anticancer activity is known world-wide.[4,5] The purpose of this research was three-fold: (1) to evaluate the potential of using microwave technology to synthesize cisplatin and, if favorable, (2) to develop a rapid, but simple, micro-scale method for general use, but specifically (3) provide a method that could facilitate the incorporation of the short-lived radioisotope, ^{195m}Pt ($t_{1/2}$ = 4.02 d)[6] into cisplatin. Achieving this goal would maximize the useful life of radiolabelled cisplatin in biological studies.[7,8]

Originally synthesized by Peyrone in 1845,[9,10] cisplatin can be synthesized using one of three basic methods, all of which use K_2PtCl_4 as the starting material. The Kauffman method uses a buffer solution of NH_4OH/NH_4Cl (pH ~ 8 – 9) as the reaction medium and the reaction is carried out in a refrigerator for 24 – 48 h.[11] Synthesis by the Lebedinskii-Golovnya[12] method (and the upgrade of this method by Kukushkin and co-workers[13]) employs an aqueous solution of NH_4OAc:KCl at reflux for 2 – 3 h. Lastly, the Dhara method is a four-step process in which K_2PtCl_4 is first converted to K_2PtI_4 *in situ*, followed by the addition of NH_4OH to produce *cis*-$[Pt(NH_3)_2I_2](s)$. Addition of $AgNO_3$ converts *cis*-$[Pt(NH_3)_2I_2](s)$ to the diaqua species, *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ to which NaCl (or HCl) is added to obtain

the final product, *cis*-$[Pt(NH_3)_2Cl_2]$.[14] World-wide, the Dhara method is the method of choice since it provides cisplatin in both high yield and high purity. Consequently, this method has been used many times to prepare ^{195m}Pt-labelled cisplatin.[6,15,16] A minor time-related drawback to this method is that it requires 2 – 3 hours to complete.

The reaction temperatures used in the Kaufman and Dhara methods are not ideally suited to carrying out a microwave-assisted synthesis of cisplatin. However, the conventional Lebedinskii-Golovnya method (1) is carried out in a single step, and is therefore suitable for a closed-system microwave procedure and (2) the reaction temperature (reflux) is easily achievable by microwave heating. Thus, the conditions of this synthesis were selected as the ideal starting point for our investigation.

Pedrick and Leadbeater[17] reported preliminary results of the microwave-assisted synthesis of cisplatin using, fortuitously, the same starting conditions as we have. (Their communication was discovered after our work was well in progress.) While their approach yields a product free of transplatin, *trans*–$[Pt(NH_3)_2Cl_2]$, their communication omits the important issues of complete characterization and purity, especially as determined by a UV-Vis spectral analysis. Analysis by UV-Vis spectroscopy is the preferred method to assay the purity of cisplatin[6,15,18-20] and the method certified by the U. S. Pharmacopeia to assure clinical grade material. Cisplatin produced by the Pedrick and Leadbeater method is contaminated by the 1:1 Magnus-type salt, $[Pt(NH_3)_3Cl][Pt(NH_3)Cl_3]$ (vide infra) that is yellow in color, has comparable aqueous solubility and identical elemental composition as cisplatin. These properties make the detection of this impurity more difficult and necessitate the development of a special purification procedure in order to obtain a pure product. The 1:1 Magnus-type salt was also detected in the product obtained by Kukushkin and co-workers (and not removed completely after two recrystallizations from HCl, as determined by Dunham and Lippard using ^{195}Pt NMR when checking the synthesis and purity of the Kukushkin preparation).[13]

EXPERIMENTAL SECTION

Materials and methods

Commercial reagent grade chemicals and solvents were used as received without further purification, except for K_2PtCl_4, which was purified by recrystallization from 1 M HCl and analyzed by UV-Vis spectroscopy, using the spectral parameters of K_2PtCl_4 (Premion) supplied by Alfa Aesar (99.99 % metals basis) as a standard of purity.

Cisplatin,[14] transplatin,[11] $K[Pt(NH_3)Cl_3]$,[21] $[Pt(NH_3)_3Cl]Cl$,[22] $[Pt(NH_3)_4]Cl_2$[23] were synthesized according to procedures reported in the literature and used as standards for our analyses.

The 1:1 Magnus-type salt was prepared by mixing equimolar amounts of $K[Pt(NH_3)Cl_3$ and $[Pt(NH_3)_3Cl]Cl$ dissolved in 0.1 M HCl. The resulting solid product was analyzed by ^{1}H NMR and UV-Vis spectroscopy.

HPLC analyses were performed using a Thermo Scientific Dionex ICS-5000 station employing a 25 µL loop, a reverse phase column (Phenomenex C8, 250 X 4.6 mm, 5 µm, 100 Å), and a UV detector operating at 220 nm. Samples were eluted in isocratic mode with HPLC grade water at 25 °C at a flow rate of 0.7 mL/min. After completing a microwave reaction, the homogenous reaction mixture was immediately sampled (10 µL) while still hot, the sample was diluted to 1.0 mL using 0.1 M HCl and was immediately analyzed by HPLC. A calibration curve for the quantitative determination of cisplatin was established as follows: six samples of pure cisplatin dissolved in 0.1 M HCl, all at different concentrations in the range 0.15 – 2.5 mM, were analyzed by HPLC; the integrated peak areas (mA min) were plotted vs the concentration of cisplatin samples (mM).

^{1}H NMR spectra in DMF-d_7 were recorded using a Jeol ECX 400 MHz instrument. Chemical shifts were referenced to the internal residual peak of DMF-d_7 at 8.03 ppm.

UV-Vis spectra were recorded using a Varian Cary 4 Bio spectrophotometer. Samples were analyzed by recording the UV-Vis spectrum of 0.1 M HCl solutions of these samples over the wavelength range

of 500 – 200 nm. A calibration curve for the quantitative determination of the amount (w/w %) of the 1:1 Magnus salt in samples of cisplatin was created as follows: standard solutions were prepared by dissolving 20.0 mg samples, containing both the 1:1 Magnus-type salt and cisplatin in the range of 0 – 10 % w/w of the 1:1 Magnus-type salt, in 25.00 mL 0.1 M HCl solution. The absorbance values at the relative minimum, A_{min}, were plotted vs the concentration of 1:1 Magnus-type salt in cisplatin samples (w/w % basis).

Microwave Syntheses

Microwave reactions were performed using a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC), having the capability of maintaining reactions at a pre-set temperature by automatically adjusting the microwave irradiation power. The maximum microwave power limit and upper pressure limit were set at the 300 W and 150 psi, respectively. Reactions were performed under magnetic stirring in 10 mL glass vessels specifically designed for microwave use. Reactions were studied as a function of the concentrations of K_2PtCl_4, KCl, and ammonium salt, nature of the ammonium salt, temperature and reaction time. These parameters were varied systematically, as discussed later in the text.

General Procedure (0.2 mmol scale). K_2PtCl_4 (83.0 mg, 0.2 mmol), KCl (30.0 mg, 0.4 mmol) and NH_4OAc (62.0 mg, 0.8 mmol) were dissolved in water (1.0 mL) in the special microwave compatible test tube. The reaction mixture was stirred magnetically and brought to a reaction temperature of 100 °C within the first minute of reaction and then held at the same temperature for 14 min longer. The reaction mixture was then chilled to 0 ˚C in an ice-water bath, inducing precipitation of a yellow solid. Complete precipitation was assured by adding 4 volumes of ethanol (EtOH). The solid was collected by centrifugation and then treated with 2.0 mL N,N-dimethylacetamide (DMA) to extract the cisplatin. Following centrifugation, the DMA solution was removed and the remaining solid was leached with an additional 0.5 mL of DMA, and the resulting solution was separated by centrifugation. The two DMA

solutions were combined, followed by the addition of 3 volumes of EtOH to precipitate the crude cisplatin product. This crude product was collected by centrifugation, washed two times with EtOH, and then dried *in vacuo* at 45 – 50 ˚C for 15 min.

Purification

The crude product, obtained as described in the Synthesis section above, was purified using one of two different purification procedures.

Method 1: Recrystallization from hot 0.1 M HCl. The crude product was dissolved in sufficient 0.1 M HCl at ~ 90 °C to produce a solution containing ~ 16 mg/mL. The resultant solution was immediately centrifuged for 20 seconds to separate any undissolved material, the yellow mother liquor was removed, and then cisplatin was precipitated by first chilling it in an ice-water bath followed by adding 3 volumes of EtOH. The solid was collected by centrifugation, washed with EtOH and then dried *in vacuo* at ~ 50˚C for 30 min. It was possible to recover 80 – 90 % of the mass of the starting crude product.

Method 2: Digestion of crude cisplatin with K_2PtCl_4. The crude product was dissolved in 0.1 M HCl at ~ 80 °C to produce a solution containing ~ 9 mg /mL. 10 μL of 0.1 M K_2PtCl_4 solution in 0.1 M HCl were added per each mg of starting crude product. The resulting red solution was digested at ~ 80 °C for 5 minutes under magnetic stirring, cooled in an ice – water bath and then crude cisplatin was quantitatively precipitated by adding 3 volumes of EtOH. The resulting solid was collected by centrifugation and then DMA was added to extract the cisplatin. After centrifugation, the yellow mother liquor was decanted from the small amount of grey – pink precipitate (Cleve salt) that had formed. Cisplatin was then re-precipitated using 3 volumes of EtOH, centrifuged, washed once with ice-cold HCl 0.1 M and once with EtOH, and dried *in vacuo* at ~ 50˚C for 30 min. It was possible to recover 75 – 80 % of the mass of the starting crude product.

RESULTS AND DISCUSSION

Optimization of the reaction conditions

In order to define the optimal reaction conditions for the microwave-assisted synthesis of cisplatin, we performed a series of reactions to evaluate the effect of the following six reaction variables: (1) reaction temperature, (2) reaction time, (3) concentration of the starting material, K_2PtCl_4, (4) scale of reaction, (5) the molar ratio of K_2PtCl_4:KCl:NH_4X, where X is the conjugate base of the parent acid (HX), molar ratios, and (6) the effectiveness of NH_4X salts as sources of NH_3.

Our initial experiments were designed to evaluate the effect of temperature and time on the course of the reaction. Preliminary reactions were carried out and the ^{1}H NMR spectra of the crude products in DMF-d_7 were recorded (Figure 1). We identified the target product, cisplatin, and other side-product chloroammineplatinum(II) complexes, by comparison with the ^{1}H NMR chemical shift values of the amminic protons for the entire series of choloroammineplatinum(II) complexes in DMF-d_7 (Numerical values in Table 1 and ^{1}H NMR spectra in Figure S1 in ESI).

Our investigation began by using a 0.2 mmol scale of K_2PtCl_4 in 1 mL of water and the same reactants and molar ratios of the reactants used in the Lebedinskii-Golovnya method,[12] *i.e.,* K_2PtCl_4:NH_4OAc:KCl = 1:4.33:5.58. The first reaction was carried out at 150 °C for 15 min, and transplatin was the main product, as indicated by the peak at 3.74 ppm in the ^{1}H NMR spectrum recorded in DMF-d_7 (Figure 1-a).

A second attempt was carried out by reducing the temperature to 100 °C and extending the reaction time to 30 min. This approach led to the formation of a considerable amount of the insoluble Magnus green salt. After cooling down the reaction mixture, a yellow precipitate formed. Both the green and yellow precipitates were recovered by centrifugation. The yellow material was extracted into DMF and separated from the green material by centrifugation. EtOH was added to the extract to produce a yellow precipitate that was washed with EtOH and dried *in vacuo*. The ^{1}H NMR spectrum of this extract,

recorded in DMF-d_7 (Figure 1-b), showed a peak at 4.90 ppm, assigned to the protons of the NH_3 ligand *trans* to the Cl ligand in $[Pt(NH_3)_3Cl]^+$, N_aH_3 in Figure 1-b. A major peak was detected at 4.21 ppm, and was assigned to the amminic protons of cisplatin. This peak showed a shoulder peak at 4.28 ppm, due to overlap with the signal of the protons of the amminic group in *cis* position to Cl in $[Pt(NH_3)_3Cl]^+$, N_bH_3 in Figure1-b. These data demonstrated that a temperature of 100 °C is an acceptable temperature to avoid the formation of transplatin, the thermodynamically favored product.[24] On the other hand, these reaction conditions seemed to enhance the formation of the tri- and tetraammineplatinum cationic species, $[Pt(NH_3)_3Cl]^+$ and $[Pt(NH_3)_4]^{2+}$, the latter of which reacts with the starting species $[PtCl_4]^{2-}$, to form the insoluble Magnus green salt.

A reaction was then carried out at 100 °C to avoid the formation of the *trans* product, and both reducing the reaction time to 15 min and the amount of ammonium acetate to 2 equivalents, to minimize the over-ammination process. No Magnus green salt formed. A major peak was detected at 4.18 ppm, and assigned to the amminic protons of cisplatin (Figure 1-c).

A final preliminary experiment was carried out using ammonium carbonate instead of ammonium acetate, to evaluate the effect of the nature of the ammonium salt on the outcome of the reaction. One equivalent of ammonium carbonate was used in order to maintain the 1:2 ratio between platinum and the ammonium ion, since carbonate is the conjugate base of a diprotic acid. The ^{1}H NMR spectrum in DMF-d_7 (Figure 1-d) showed a peak at 4.88 ppm, assigned to the protons of the amminic group in a position *trans* to Cl in $[Pt(NH_3)_3Cl]^+$, N_aH_3 in Figure 1-d. A major peak was detected at 4.20 ppm, and assigned to the amminic protons of cisplatin. This peak showed a shoulder peak at 4.26 ppm, as a result of the overlap with the signal of the protons of the amminic group in the position *cis* to Cl in $[Pt(NH_3)_3Cl]^+$, N_bH_3 in the Figure 1-d.

These first experiments suggested that the formation of transplatin as a side-product is independent of the nature of the ammonium salt, but is related strictly to the reaction temperature. Nevertheless, the

nature of the ammonium salt affects the rate of the ammination, leading to the formation of a different amount of the $[Pt(NH_3)_3Cl]^+$ species.

In the following step of this project HPLC turned out to be a powerful instrumental method for the investigation of the microwave-assisted synthesis of cisplatin performed under different reaction conditions. HPLC analyses allowed us to determine the presence of charged Pt(II) species (that eluted after 2.5 – 3.0 min as a broad left-tailed peak), cisplatin (eluting after 4.0 min), and transplatin (eluting after 4.4 min) (Figure S2 in ESI). Moreover, HPLC analyses allowed us to calculate the yield of cisplatin obtained under different reaction conditions. The calibration curve for the quantitative determination of cisplatin was linear over the range 0.15 – 2.50 mM and is described by the equation: $y = 68.757\ x - 0.318$ ($r = 0.9987$), wherein y is the integrated peak area (mA min) and x the concentration of cisplatin in samples (mM) (Figure S3 in ESI). The regression analysis equation obtained from the calibration curve was used to determine the concentration of cisplatin in 10 μL samples of fresh reaction mixtures diluted to 1.0 mL with 0.1 M HCl. These data were then used to calculate the yield of cisplatin for the reaction under examination.

In a series of reactions, the amount of K_2PtCl_4 (0.2 mmol) and its concentration (0.2 M) were kept constant, as well as the reaction temperature (100 °C) and reaction time (15 min). The nature and the amount of the ammonium salt used (NH_4X or $(NH_4)_2X$) and the amount of KCl were varied systematically to investigate the effects of these variations on the yield of cisplatin. In the first part of the microwave experiment the irradiation power, on average, reached 40 – 50 W, achieving the target temperature of 100 °C. The temperature was maintained at 100 ± 4 °C, and the irradiation power decreased to 1 – 5 W in order to keep the temperature constant. The pressure increased in the first part of the experiment to 35 – 50 psi, and then remained in this range for the remaining time (Figure S4 in ESI).

In general, low KCl concentrations, in the range 0 – 0.2 M, led to the formation of a dark precipitate, possibly Pt^0, as reported by Ghedini and co-workers.[25] Formation of the dark precipitate was not observed for concentrations of KCl in the range of 0.4 – 1.2 M.

The bar graph (Figure 2, numerical values in Table S1 in ESI) shows the predicted yields of cisplatin (*i.e.,* % yield based on HPLC analyses) as a function of the molar ratio of NH_4^+:KCl for four different NH_4X salts: Ammonium Acetate (NH_4OAc), Ammonium Carbonate ($(NH_4)_2CO_3$), Ammonium Bicarbonate (NH_4HCO_3), and Ammonium Hydroxide (NH_4OH). The ratios were varied from 2:2 to 4:6.

When NH_4OAc was used as the ammine source (data in blue in the graph) and using a stoichiometric NH_4OAc:K_2PtCl_4 molar ratio of 2:1, low yields were obtained: 18, 11 and 10 % in the presence of 2, 4 and 6 equivalents of KCl, respectively. The highest yields were obtained in presence of 2 equivalents of KCl and 3 and 4 equivalents of NH_4OAc, yielding 52 and 54 % cisplatin, respectively. When the amount of KCl was increased, a general decrease in the yield of cisplatin occurred, yielding 44, 24, 26 and 29 % cisplatin for 3:4, 3:6, 4:4 and 4:6 NH_4OAc:KCl molar ratios, respectively. A slight countertrend of the yield was observed passing from a 4:4 to 4:6 NH_4OAc:KCl molar ratio. Although a lower concentration of KCl seemed to provide a higher yield of cisplatin in these experiment with NH_4OAc, the reaction was not performed in presence of KCl in amounts lower than 2 equivalents in order to avoid the formation of the dark precipitate reported above. On the other hand, the reaction with a 6:2 NH_4OAc:KCl molar ratio (Data not reported in the graph) led to a decrease in yield to 40 %. This suggests that a higher excess of NH_4OAc does not increase the yield of cisplatin, but rather enhances the formation of the $[Pt(NH_3)_3Cl]^+$ and $[Pt(NH_3)_4]^{2+}$ species.

When $(NH_4)_2CO_3$ was used as the source of NH_3 (data in violet in the graph), with a stoichiometric amount of $(NH_4)_2CO_3$, *i.e.* 1:1, cisplatin was obtained in 11, 23, and 23 % yield in the presence of 2, 4, and 6 equivalents of KCl, respectively. These results suggest that in the presence of $(NH_4)_2CO_3$ the

formation of tri- and tetrammine products is enhanced, in agreement with the results of preliminary NMR experiments. By increasing the amount of KCl, the yield of cisplatin increased, suggesting that a higher concentration of chloride ions in solution counterbalances the over-ammination trend in favor of the formation of cisplatin. Performing the reaction in the presence of 1.5 equivalents of $(NH_4)_2CO_3$, (i.e., 3 equivalents of ammonium ion), we obtained 25, 12, and 23 % yields of cisplatin in the presence of 2, 4, and 6 equivalents of KCl, respectively. Using 2 equivalents of $(NH_4)_2CO_3$, i.e., 4 equivalents of ammonium ion, we obtained 33, 35 and 25 % yield of cisplatin, in the presence of 2, 4, and 6 equivalents of KCl, respectively. These trends are difficult to rationalize, but might be understood in terms of the diprotic nature of the CO_3^{2-} ion which could give rise to more complex equilibria in solution.

Using a stoichiometric amount of NH_4HCO_3 as the ammonolysis agent (data in orange), we obtained cisplatin yields of 21, 30 and 42 %, in presence of 2, 4, and 6 equivalents of KCl, respectively. Also in this case, it seems that the use of NH_4HCO_3 enhances the formation of tri- and tetraammine products, and that a higher concentration of chloride ions in solution can be used to shift the reaction in favor of the formation of the target diammine product. Using an excess of NH_4HCO_3 (3 equivalents) led to higher yields of cisplatin than the stoichiometrical ratio of NH_3:Pt: 22, 17, and 14 % in the presence of 2, 4, and 6 equivalents of KCl, respectively. Under stoichiometric conditions it seems that part of the ammonia in solution was consumed in the formation of tri- and tetraammine complexes, leaving some starting material unreacted. The use of an excess of this ammonium salt can provide an additional amount of ammonia that can react with unreacted starting material, and lead to an increase in the yield of cisplatin. When a higher excess of NH_4HCO_3 was used, low yields of 3, 6 and 4 %, were obtained in the presence of 2, 4, and 6 equivalents of KCl, respectively. In this last set of reactions, the reaction mixtures appeared as pale yellow solutions with some green precipitate, indicating that a high excess of

NH_4HCO_3 enhanced the formation of $[Pt(NH_3)_4]^{2+}$ species that can react with unreacted $[PtCl_4]^{2-}$, forming the Magnus green salt.

Finally, reactions were performed using NH_4OH as the ammonolysis agent (green bars in the graph). Using a stoichiometric amount of NH_4OH, the yields of cisplatin were 34, 21 and 33 %, in the presence of 2, 4, and 6 equivalents of KCl, respectively. Using 3 equivalents of NH_4OH we obtained 50, 17 and 19 % yield of cisplatin, in the presence of 2, 4, and 6 equivalents of KCl, respectively. Finally, using 4 equivalents of NH_4OH, yields of cisplatin of 44, 34 and 25 % were obtained in the presence of 2, 4, and 6 equivalents of KCl, respectively. These data indicated that NH_4OH is a good ammonolysis agent, although using NH_4OAc led to better yields.

Although the ammonolysis of K_2PtCl_4 to form cisplatin can be represented by the overall equation (Eqn 1):

$$K_2PtCl_4 + 2\,NH_3 \Rightarrow \textit{cis}\text{-}[Pt(NH_3)_2Cl_2] + 2\,KCl \qquad (1)$$

the reaction process is exceedingly more complex than the equation suggests because of kinetic and thermodynamic factors, competing reactions, and differences in the solubility of products. The complete sequence of reactions can involve different species, starting with $[PtCl_4]^{2-}$ and products formed by sequentially replacing Cl^- by NH_3, ending up with the tetraammineplatinum(II) cation, $[Pt(NH_3)_4]^{2+}$. In addition, platinum-based salts can be formed by combinations of the cationic and anionic choroammineplatinum(II) species: Magnus green salt, $[Pt(NH_3)_4][PtCl_4]$; Cleve salt, $[Pt(NH_3)_3Cl]_2[PtCl_4]$; 1:1 Magnus-type salt, $[Pt(NH_3)_3Cl][Pt(NH_3)Cl_3]$; and the 2:1 Magnus-type salt $[Pt(NH_3)_4][Pt(NH_3)Cl_3]_2$ (Figure 3)**.** These species are not in equilibrium, but each step of this reaction sequence is reversible and, at constant temperature, its reversibility is a function of the concentration of Cl^- and NH_3.[26] Higher Cl^- concentration constrains the product range to species higher in the number of Cl^- ligands while higher NH_3 concentration skews the product range to species containing a higher

number of NH_3 ligands. Thus, there is an optimal NH_3:Cl^- ratio that maximizes the yield of cisplatin in this range of possible products.

The yield of cisplatin changes with the nature of the ammonium salt used, NH_4X, since the basicity of X^-, the conjugate base of a weak acid HX, affects the ease/extent of release of NH_3 and thus the NH_4^+/NH_3 equilibrium. More studies are needed to establish a possible quantitative relationship. In this context, anions of strong acids have virtually no effect on the release of NH_3 and, as was observed, NH_4Cl and $(NH_4)_2SO_4$ do not function as ammoniating agents (data not shown).

After determining the optimal conditions of synthesis in terms of NH_4^+:Cl^- stoichiometric ratios and the nature of the ammonium salt, we tried to refine the reaction temperature and time in order to maximize the yield of cisplatin. At 80˚C and 90 ˚C, a 15 min-long reaction was incomplete, resulting in reduced yields of cisplatin and products rich in chloride (i.e., K_2PtCl_4 and $K[Pt(NH_3)Cl_3]$). At 120˚C, the *trans*-$[Pt(NH_3)_2Cl_2]$ isomer was first detected indicating that the thermodynamically more stable isomer was emerging. This finding is in agreement with our earliest results in which we found that at 150˚C *trans*-$[Pt(NH_3)_2Cl_2]$ was the exclusive product and can be regarded as the first step for the development of microwave-assisted procedures for the synthesis of ^{195m}Pt-labelled *trans* dichlorodiamineplatinum(II) complexes for pre-clinical and clinical studies.[27]

We also investigated the influence of reaction time. Reducing the reaction time to 12.5, 10, 7.5 and 5 min, the HPLC analyses showed the predicted yield decreasing to 47, 45, 38 and 37 %, respectively. On the other hand, extending the reaction time to 17.5 and 20 min, the predicted yield decreased to 43 and 33 %, respectively, probably because the longer reaction time promoted further ammination of cisplatin to form the cationic tri- and tetraammine complexes. These data, summarized in Figure 4, confirmed that 100 °C and a 15 min reaction time are the optimal reaction conditions.

Purification

Once we set the ideal conditions for the microwave-assisted synthesis of cisplatin on 0.2 mmol scale, we established guidelines for a rapid, efficient, and easy isolation procedure that yields a highly pure cisplatin product. DMF and DMA were separately evaluated as extractants of cisplatin in developing the process permitting the isolation of the crude cisplatin, as cited at the end of the General Procedure (see above). When DMF was used, a typical UV-Vis spectrum showed (Table 2) a λ_{max} = 301 nm, a λ_{min} = 253 nm, a A_{max}/A_{min} ratio of 1.9 and ε_{301} = 124 M^{-1} cm^{-1}. By using DMA for the extraction of cisplatin instead of DMF, the purity of the crude product was enhanced, yielding a λ_{max} = 301 nm, a minimum at 247 nm, a A_{max}/A_{min} ratio of 2.6 and ε_{301} = 122 M^{-1} cm^{-1} in a typical UV-Vis analysis. In both cases, the crude product required further purification since the spectral parameters of the crude material did not match the spectral criteria for pure cisplatin.[6]

The color and the solubility of these crude cisplatin products are similar to those of pure cisplatin, although the UV-Vis analysis clearly indicated that substantial impurities were present. Analysis of these crude cisplatin products (and a product synthesized by the Lebedinskii-Golovnya method) by ^{1}H NMR, confirmed the presence of the ions, $[Pt(NH_3)_3Cl]^+$ and $[Pt(NH_3)Cl_3]^-$ in these products. Given this information and the report by Dunham and Lippard who identified (by ^{195}Pt NMR) the presence of the 1:1 Magnus-type salt as an impurity in cisplatin prepared by the method of Kukushkin, and co-workers, it was clear that the 1:1 Magnus-type salt was undoubtedly the major contaminant in the microwave-assisted preparations of cisplatin.[13] In order to determine the quantity of 1:1 Magnus-type salt in crude cisplatin, we established a calibration curve plotting the A_{min} values of UV-Vis spectra vs the concentration of 1:1 Magnus-type salt in cisplatin samples (w/w %). This calibration curve is linear over the range 0 – 10 %: $y = 0.0048\,x + 0.0679$ ($r = 0.9910$), where y is A_{min} and x the concentration of 1:1 Magnus-type salt in cisplatin sample (%, w/w) (Figure S5 in ESI). The regression analysis equation

obtained from the calibration curve indicated an average concentration of 1:1 Magnus-type salt of ≤ 10 % w/w in the crude cisplatin samples.

Two methods were developed to provide a highly pure final product. When the crude product was purified by recrystallization from hot 0.1 M HCl (Method 1 in the experimental section), UV-Vis measurements showed the following average values: λ_{max} = 301 nm, λ_{min} = 247 nm, A_{max}/A_{min} ratio = 5.0, and ε_{301} = 131 $M^{-1}cm^{-1}$. These values matched the spectral purity requirements reported for cisplatin in the literature[6] and in the U. S. Pharmacopeia. It was possible to recover 80 – 90 % of the mass of the starting crude product, with a final cisplatin yield of 47 %.

Using purification method 2, reported in the experimental section, the crude cisplatin was digested in the presence of K_2PtCl_4 to fragment the 1:1 Magnus-type salt and selectively form the less soluble Cleve salt, $[Pt(NH_3)_3Cl]_2[PtCl_4]$. UV-Vis measurements showed the following average values: λ_{max} = 301 nm, λ_{min} = 246 nm, A_{max}/A_{min} ratio = 4.6, and ε_{301} = 132 M^{-1} cm^{-1}, matching the spectral purity requirements reported in the literature[6] and in the U. S. Pharmacopoeia. It was possible to recover 80 – 85 % of the mass of the starting crude product, with a final cisplatin yield of 43 %.

The UV-Vis measurements for the products obtained using the purification procedures reported herein and the comparison with reference values from the literature are reported in Table 2. A typical UV-Vis spectrum of pure cisplatin is reported in Figure S6 in ESI. The purity of the final product was checked also by HPLC, detecting just the peak belonging to cisplatin at 4.0 min (Figure S7 in ESI).

Downscale to 0.05 mmol

Finally, microwave-assisted syntheses of cisplatin were carried out on a 0.05 mmol scale to develop a method that would facilitate the incorporation of ^{195m}Pt into cisplatin for biological studies. A scale of 0.05 mmol of K_2PtCl_4 is very appropriate because this scale is comparable to the amount of ^{194}Pt (≥ 10 mg) that has been typically irradiated in a reactor to produce ^{195m}Pt.[6,8] Results indicated that the highest

yield of cisplatin, 43 %, was obtained using a concentration of starting K_2PtCl_4 of 0.1 M, while using a concentration of 0.05 or 0.2 M, the yield decreased to 32 and 24 %, respectively (Figure 5). This fact could be due to the possibility of an incomplete reaction at low concentrations (0.05 M), and to surface effects and alterations of reaction rates in small volumes (250 µl in the case of 0.2 M concentration).[16] Thus, a concentration of starting K_2PtCl_4 of 0.10 M appeared to be the optimal concentration for reactions carried out at 100 ˚C for 15 min on a 0.05 mmol scale. Nevertheless, under these conditions a dark precipitate formed, similar to what we observed in other cases in which we used a low concentration of KCl.[25] We optimized the reaction conditions increasing the amount of KCl to 5 equivalents, obtaining a HPLC predicted yield of 57 %. After purification, we collected the final pure product in a 46 % yield, comparable to the yield of the process proposed by Tinker and co-workers, affording 43 % yield.[16]

Although the present procedure does not allow yields to be obtained comparable to that of the Dhara method and the Dhara method-based microscale synthesis of ^{195m}Pt-labeled cisplatin (affording a high yield of ~ 70 %, on a 0.05 mmol scale),[6,15] it saves a considerable amount of time compared to Dhara method, since the whole process, starting with K_2PtCl_4 and ending with a pure and dried cisplatin, requires *ca.* 80 minutes instead of 2 – 3 h for the Dhara method.

This saving of time is important for the incorporation of the short-lived radioisotopes, ^{191}Pt ($t_{1/2}$ = 3.0 d), ^{193m}Pt ($t_{1/2}$ = 4.33 d)[28] and ^{195m}Pt ($t_{1/2}$ = 4.02 d),[6] since it could maximize the useful life of radiolabelled cisplatin in biological studies. This aspect would be especially crucial if one were to consider incorporating ^{197}Pt into cisplatin (^{197}Pt is a β, γ-emitter, $t_{1/2}$ = 20 h and has a specific activity considerably higher than ^{195m}Pt).[6]

CONCLUSION

We have determined (1) the ideal conditions of the microwave-assisted synthesis of cisplatin in terms of reaction time, temperature, nature of ammonium salt, NH_4X, and NH_4X:KCl ratio to maximize the yield and (2) two procedures to purify the reaction products in order to be suitable for preclinical and, possibly, clinical tests.

In general, better yields were achieved by using an excess of an ammonium salt, although higher amounts shift the reaction course in favor of the formation of tri- and tetrammine products. This tendency was partially balanced by increasing the concentration of KCl in solution. The best amminolytic agent was ammonium acetate, providing the highest yields using a K_2PtCl_4:NH_4OAc:KCl molar ratio of 1:4:2 on a 0.2 mmol scale and 1:4:5 on a 0.05 mmol scale.

We also investigated the effect of the reaction temperature. A temperature of 100 °C is the ideal temperature for the synthesis of cisplatin, while at higher temperatures, transplatin begins to form and, at 150 °C, it is the major product. This result suggests that a microwave-assisted approach using higher reaction temperatures may indeed provide a general method for preparing *trans* dichlorodiamineplatinum(II) complexes directly from K_2PtCl_4 in a single step.

Although the time saved with this microwave-assisted procedure is probably not sufficient to compensate for the difference in yield that can be obtained using the Dhara method, in order to maximize the life of ^{195m}Pt incorporated into cisplatin, this work can be regarded as a potential starting point to synthesize other *cis*- and *trans*- dichlorodiamineplatinum(II) complexes in a very short time frame.

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TABLES

Table 1. ^{1}H NMR chemical shifts of chloroammineplatinum(II) complexes in DMF-d_7.

Complex	**NH_3 ^{1}H NMR chemical shift (ppm)**
$K[Pt(NH_3)Cl_3]$	3.61
cis-$[Pt(NH_3)_2Cl_2]$	4.17
trans-$[Pt(NH_3)_2Cl_2]$	3.74
$[Pt(NH_3)_3Cl]Cl$	4.89 (N_aH_3, 3H), 4.27 (N_bH_3, 6H)
$[Pt(NH_3)_4]Cl_2$	4.70
1:1Magnus-type salt	4.64 (N_aH_3, 3H), 4.19 (N_bH_3, 6H), 3.61 (N_cH_3, 3H)

Table 2. UV-Vis Measurements for the Determination of the Purity of Cisplatin.

	λ_{max} (nm)	λ_{min} (nm)	A_{max}/A_{min} (ratio)	ε_{301} (M^{-1} cm^{-1})
Standard of Purity[6]	301	246	≥ 4.5	131 ± 2
Crude product from DMF	301	253	1.9	124
Crude product from DMA	301	247	2.6	122
Recrystallization from hot 0.1 M HCl	301	247	5.0	131
Digestion with K_2PtCl_4	301	246	4.6	132

FIGURES

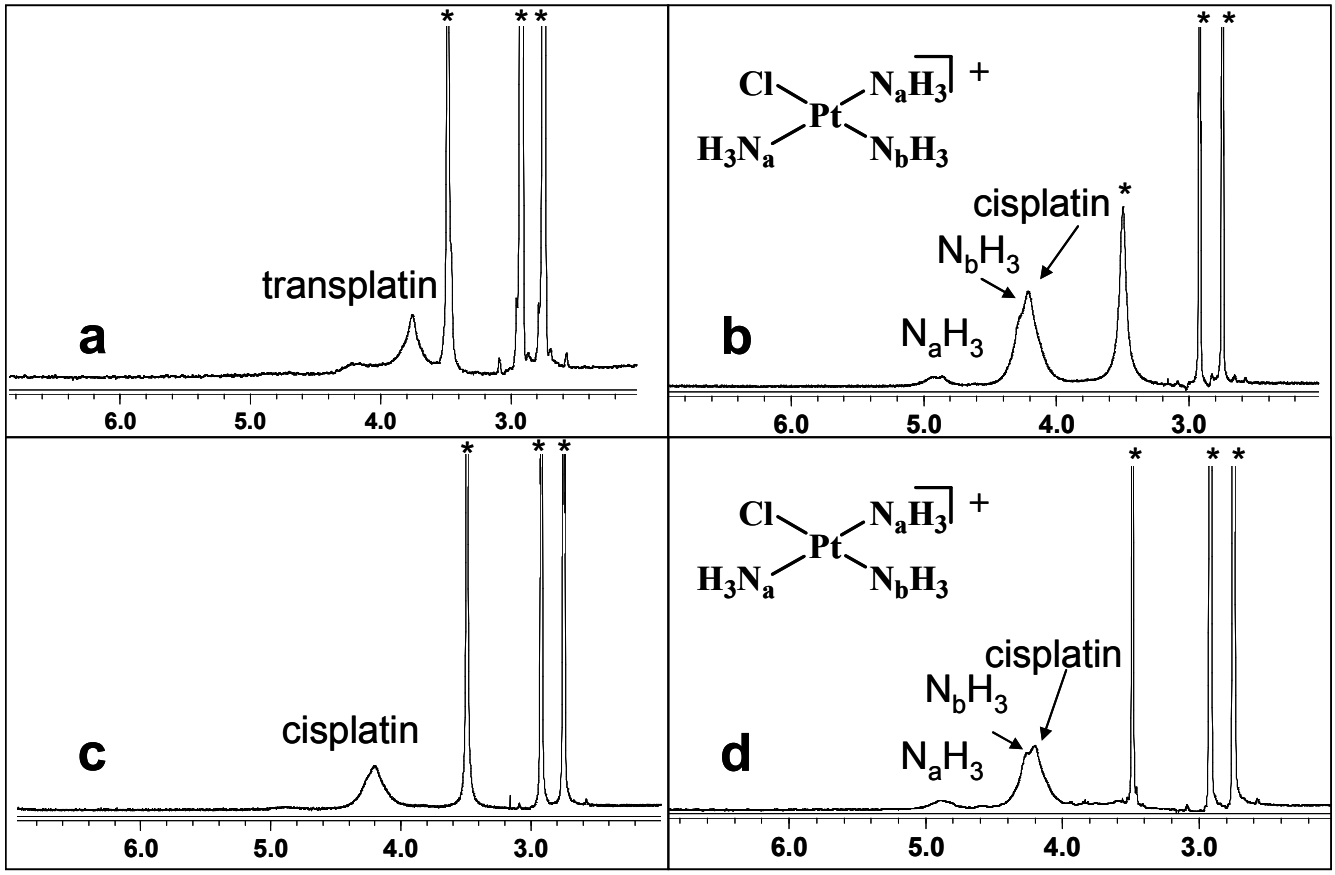

Figure 1. ^{1}H NMR spectra in DMF-d_7 and proton assignments of the crude products of the reactions carried out at a) 150 °C, 15 min; b) 100 °C, 30 min, c) 100 °C, 15 min using ammonium acetate, d) 100 °C, 15 min using ammonium carbonate. Asterisks indicate residual water and $HCON(CH_3)_2$ peaks.

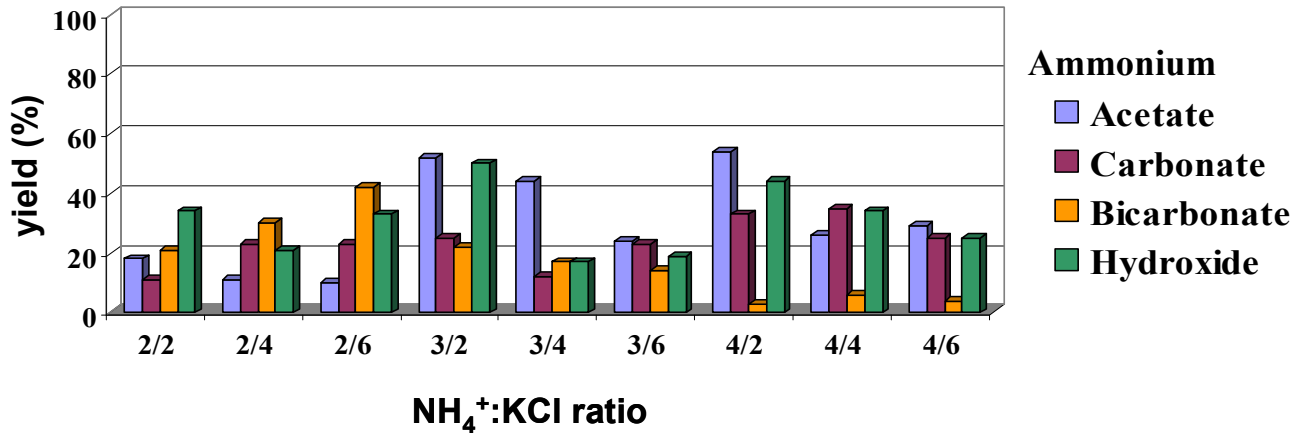

Figure 2. Bar graph of the predicted yields of cisplatin (*i.e.,* % yields based on HPLC analyses) as a function of the molar ratio of NH_4X:KCl for four different NH_4X salts: Ammonium Acetate (NH_4OAc), Ammonium Carbonate ($(NH_4)_2CO_3$), Ammonium Bicarbonate (NH_4HCO_3), and Ammonium Hydroxide (NH_4OH).

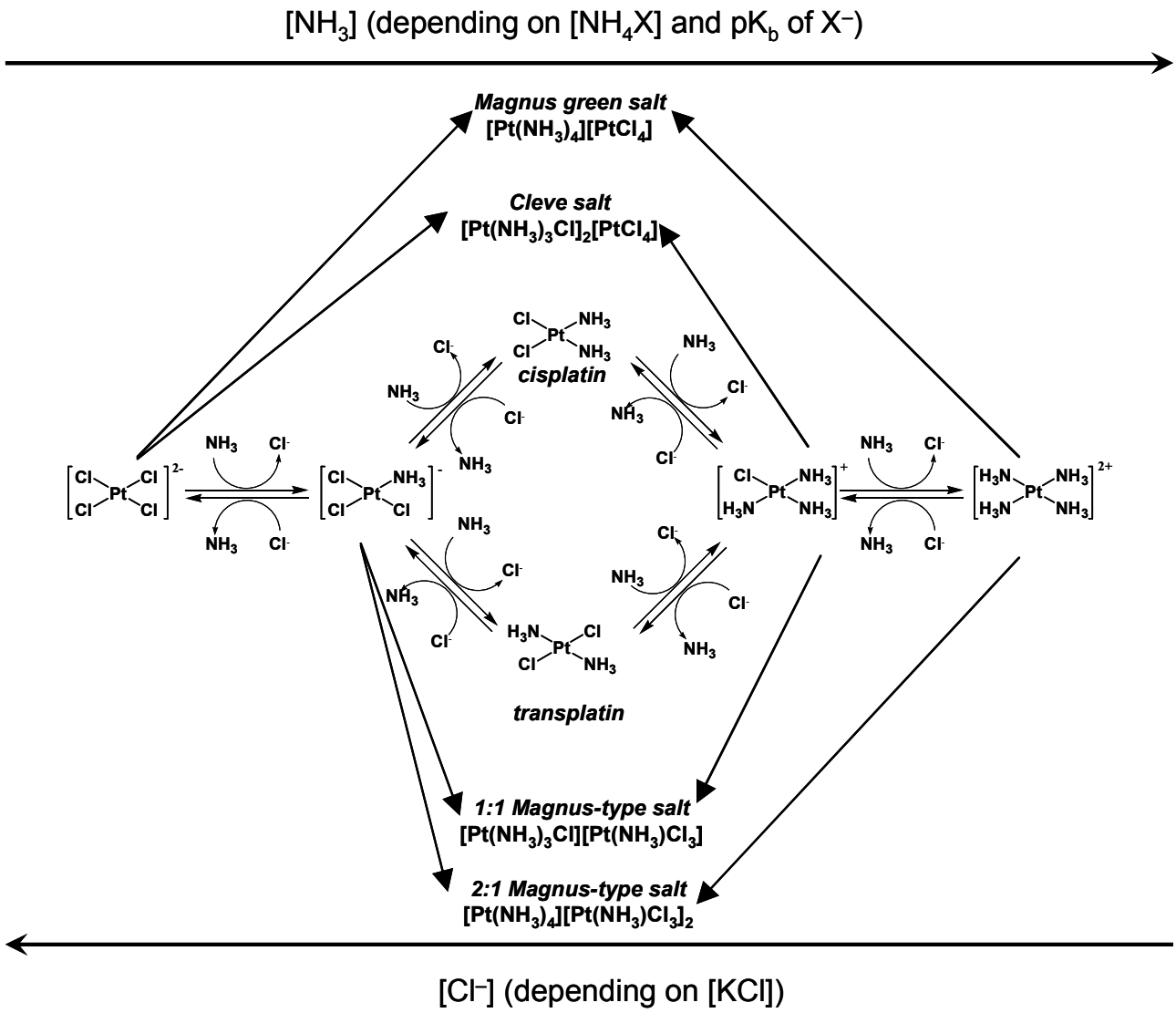

Figure 3. Scheme of the complete sequence of reactions and species involved in the microwave-assisted synthesis of cisplatin and influence of $[Cl^-]$ and $[NH_3]$ on the products trend.

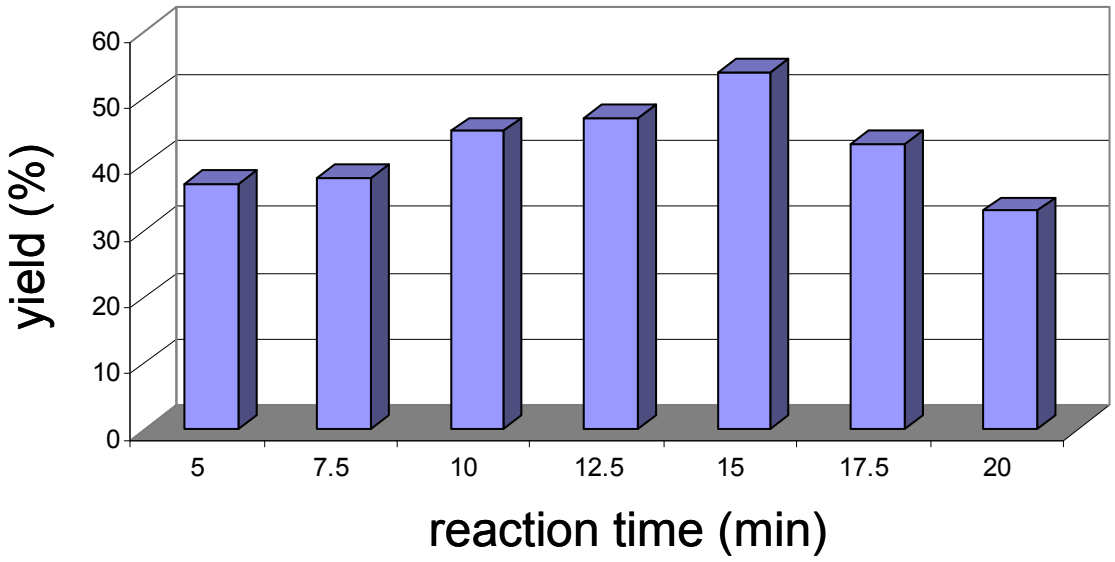

Figure 4. HPLC predicted % yields of cispaltin vs reaction time in min.

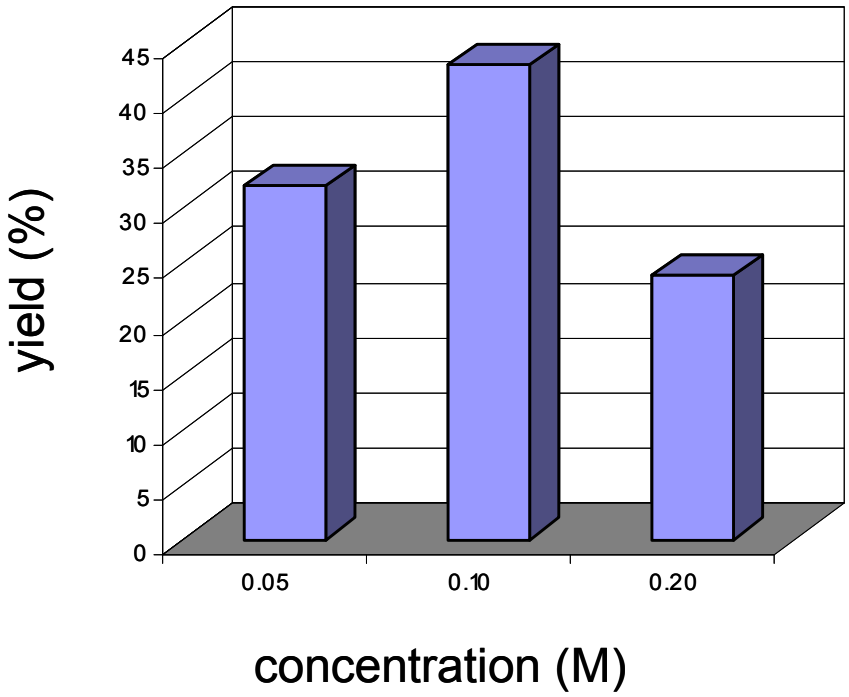

Figure 5. Variation of % yield of cisplatin on a 0.05 mmol synthetic scale vs. the starting K_2PtCl_4 concentration with a K_2PtCl_4:NH_4OAc:KCl molar ratio of 1:4:2.

TABLE OF CONTENTS SYNOPSIS AND GRAPHIC

A rapid one-step microwave-assisted synthesis of cisplatin and two purification methods were developed. The process requires about 80 min and could be used to incorporate short-lived Pt radionuclides into cisplatin.