Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, Nos 10–12, pp. 1009–1018, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00

Solution chemistry and analytical characterization of ormaplatin*

S.E. NORTHCOTT,† J.G.D. MARR,‡ S.L. SECREAST,‡ F. HAN† and J. DEZWAAN†§

† *Physical and Analytical Research and* ‡ *Control Development Division, The Upjohn Company, Kalamazoo, MI 49001, USA*

Abstract: Ormaplatin is a racemic, platinum(IV), anti-cancer drug which is currently involved in phase 1 clinical trials. Characterizing the purity of ormaplatin represents an analytical challenge for several reasons. These include the lack of solution stability of ormaplatin, its process impurities and solution decomposition products, the strong concentration and pH dependence of various decomposition pathways and solution equilibria and the absence of chromatographic reproducibility. Two independent types of aqueous decomposition pathways have been observed. The first pathway involves the generation of soluble Pt^{IV} containing species which remain in equilibrium with the parent material in solution. The second pathway involves the generation of Pt^{II} containing materials which tend to be less soluble. The most interesting material in this latter class is probably the chloride bridged mixed valence material which has been characterized structurally by X-ray diffraction. Liquid chromatography and sample handling procedures have been developed which allow for the accurate determination of bulk drug purity, as well as, an understanding of numerous reversible equilibria.

Keywords: *Ormaplatin; liquid chromatography; degradation; kinetics; equilibria.*

Introduction

Ormaplatin, trans (±) 1,2-diaminocyclohexane platinum (IV) tetrachloride, is an anti-cancer agent that is currently involved in clinical trials in the USA. Although similar in composition to other platinum based anti-cancer agents, ormaplatin contains platinum in the +4 oxidation state. This change in composition may provide a material which is better tolerated with reduced toxicity and increased efficacy [1–7]. Recent results do indicate that unexpected chemical transformations of ormaplatin are observed *in vitro* in certain cell lines [8].

Several liquid chromatographic systems have been reported for the analysis of ormaplatin [9, 10]. For the study of biotransformations, including ligand substitution reactions, a two-column approach has been effective [11]. For the analysis of simple aqueous or saline solutions of bulk drug, however, fairly simple procedures have been developed. Some of these systems, when coupled with a platinum selective detector, were shown to completely elute all of the platinum containing species, which were encountered in aqueous solutions of ormaplatin [12]. The platinum selective detection system also demonstrated the presence of platinum in a series of materials formed as a function of time in most solutions of ormaplatin analysed.

Analysis of aqueous ormaplatin solutions indicated that the formation of two primary decomposition products always occurred soon after the material was dissolved. Despite the great care used to minimize decomposition by analysing solutions immediately after preparation, a considerable degree of variability was observed in purity determinations conducted using different samples of the same synthetic lot of ormaplatin. Because the variation involved not only the relative peak areas observed but also which peaks were observed, additional investigations were conducted to understand the experimental variables which must be controlled to obtain results which were truly representative of the bulk drug composition. These studies demonstrated that upon aqueous dissolution, a complex series of chemical changes and equilibria are initiated in ormaplatin solutions. Some of these chemical

* Presented at the "Third International Symposium on Pharmaceutical and Biomedical Analysis", April 1991, Boston, MA, USA.

§ Author to whom correspondence should be addressed.

transformations occurred rapidly and appeared to reach an equilibrium. The rate of formation of these materials and their approach to equilibrium seemed to be concentration dependent. The other set of reactions appeared to occur on a slower time scale and to involve reduction of Pt^{IV} to Pt^{II}. Although under certain circumstances an equilibrium appeared to be established, some of the platinum containing species formed in solution are only sparingly soluble in water and slowly (over the course of days) begin to precipitate and therefore to reduce the level of platinum in solution.

Although the chemistry of ormaplatin in aqueous solution is still not fully understood, some of the process impurities and aqueous decomposition products have been identified. In addition to this, conditions have been established which allow samples of ormaplatin bulk drug to be analysed in a reproducible manner and in a manner which accurately reflects the actual composition of this material in the solid state.

Experimental

Chromatographic systems

The chromatography was performed with a Spherisorb ODS2 column (250 × 4.6 mm i.d., 5 μm, Alltech Associates, Deerfield, IL) using the conditions described previously [12] which employed a mobile phase of acetonitrile–water (5:95, v/v). Although this system was satisfactory for following the rapid and reversible solution chemistry, certain process impurities could not be reliably resolved from the major component using this system and therefore a more reliable method was required for purity assessment.

The ion chromatographic determinations of $PtCl_6^{2-}$ were performed using a Dionex Model 4000i pump and a cation exchange column (HPIC-AS5A column, Dionex Corporation, Sunnyvale, CA). An isocratic mobile phase consisting of 0.2 M $NaClO_4$ and 40 mM HCl was used at a flow rate of 1 ml min^{-1}. Detection was by UV absorbance at 254 nm using a Spectraflow, Model 783 detector (Kratos Analytical Instruments, Ramsey, NJ). The ion chromatographic determinations of Cl^- were made using a Dionex AS3 anion exchange column according to procedures described in detail in ref. 13.

X-ray diffraction

X-ray diffraction data were collected on a Nicolet $P2_1$ X-ray diffractometer controlled by a Harris computer at low temperature (−150°C) with monochromatized MoK_α radiation (0.7107Å), using a crystal mounted on a glass fibre. All 2174 unique reflections were measured to a $2\theta_{max}$ of 65° for Laue group $2/m$. The $\theta/2\theta$ step-scan technique was used with a scan speed of 2° min^{-1} and a scan width >3.4°. Eight reflections periodically monitored showed no trend towards deterioration. Standard deviations in the intensities were approximated from the following equation:

$$\sigma^2 = \sigma^2(I)_{\text{counting statistics}} + (0.0086I)^2 \quad (1)$$

where the coefficient of I was calculated from the variation in the intensities of the monitored reflections.

Materials

The ormaplatin used in these studies was obtained from the National Cancer Institute (Bethesda, MD, USA) while dexormaplatin was prepared in house by the same synthetic procedure using trans-(*R*,*R*)-1,2-cyclohexanediamine with optical purity of >99.5%. The $PtCl_6^{2-}$ was obtained from Aldrich Chemical Company (Milwaukee, WI). Sodium perchlorate was obtained from GFS Chemical Company (Columbus, OH). Acetonitrile (HPLC grade) was obtained from Burdick and Jackson (Muskegon, MI). All water was obtained from a Milli-Q Water System (Millipore Corporation, Milford, MA) and was 18 MΩ quality. The di-hydroxy analogue of ormaplatin was synthesized in house according to the procedure given in ref. 14.

Analytical quantities of DP1 and DP2 were isolated from aqueous solutions of ormaplatin (5 mg ml^{-1}) that were allowed to age on a benchtop. DP1 was isolated using Microsorb C-18 column (250 × 21.4 mm i.d., 5 μm, Rainin Instrument Company Inc., Woburn, MA). Up to 30 ml of the aged ormaplatin solutions were loaded onto the column and eluted with 2% acetonitrile in water. After collecting the DP1 peak the column was washed with acetonitrile–water (25:75, v/v) before the next injection. DP2 was isolated using the Spherisorb ODS2 column described above. A Valco injector with a 2.7 ml loop was used to load the column and the material was eluted with acetonitrile–water (25:75, v/v) at a

flow rate of 2 ml min^{-1}. Purities of 98%, based on peak areas, were found for both materials isolated in this manner using an analytical chromatographic system suitable for ormaplatin.

Results and Discussion

Liquid chromatography

As part of the investigation to improve the selectivity of a non-chiral chromatographic system for ormaplatin and its (*R,R*) enantiomer, dexormaplatin, the analytical potential of a number of columns was examined. A variety of binary mobile phases were used for each column investigated. These mobile phases did not contain any buffers, as there have been reports in the literature [15–17] of the potential for nucleophilic components in the mobile phase to react with Pt^{II} compounds during chromatography. However, acetonitrile was included in the range of organic modifiers tested, both as a reference for comparison with the original C-18 separation, and as an example of a likely on-column organic reactant [15–17].

A variety of separations were obtained for different mobile phase combinations and for different column types. Some normal phase separations were also attempted, but as the majority of the UV spectra obtained from the peaks that eluted did not resemble those in the reference data set, these separations were not investigated further. These results seem to give some support to the ideas of on-column solute–solvent interactions expressed in refs 15 and 16.

Of the reversed-phase separations that were observed, those based on the Dynamax Microsorb cyano column (250 × 4.6 mm i.d., 5 μm, Anachem, Luton, UK) were chosen for further investigation, as being the most likely to involve different separation mechanisms than those observed on the original C-18 column. Following a comparison of the different selectivities of methanol, tetrahydrofuran and acetonitrile for this compound and its potential process impurities and decomposition products, optimal separation was achieved using a 100% aqueous mobile phase and a Dynamax Microsorb 3-μm cyano (30 × 4.6 mm i.d.) guard column. This mixed particle size approach provided a better separation than either a 3-μm or a 5-μm packed column alone.

The chromatograms in Fig. 1 illustrate the selectivity of the C-18 and cyano systems for omaplatin and its two primary aqueous decomposition products which will be referred to as DP1 and DP2 throughout the remainder of this text. Given in Table 1 are the retention times of these decomposition products relative to ormaplatin using the HPLC columns indicated and the respective mobile phases. From these data it is apparent that the selectivities obtained are highly dependent on the column used.

Analytical sample stability

When ormaplatin was dissolved in water a complex series of chemical transformations were initiated. Some of these processes occurred quite rapidly and involved simultaneous decreases in the solution pH, formation of decomposition products DP1 and DP2 and a continuous buildup of Cl^-. These changes appeared to proceed until an equilibrium with ormaplatin was established. Other processes occurred more slowly and resulted in the appearance of precipitates in the solutions over the course of days and weeks. Both of these decomposition routes have an impact on the analysis of ormaplatin bulk drug and have been studied in detail.

Rapid equilibria

Figure 2 shows the changes in concentration that occur in ormaplatin, DP1 and DP2 in an aqueous ormaplatin solution at a concentration of 1 mg ml^{-1} as a function of time. The data presented in Fig. 3 clearly demonstrate that the initial rate at which these processes occur depends strongly on the initial ormaplatin concentration of the solutions used. The data in Fig. 2 were collected on a sample that was prepared at the end of the working day and then analysed overnight using an autosampler. The data also indicate that an equilibrium was approached between 10 and 16 h with an increase in chloride ion concentration from 0.2 to 2.5 mM and a decrease in pH from 4.7 to 3.7. When the laboratory lights were turned on after 16 h, however, the reactions appeared to be driven further, demonstrating the influence of light on this system. The tendency of the various components to approach a plateau with time under constant conditions indicates that a quasi-equilibrium situation is established. Further evidence for the existence of solution equilibria and at least partial reversibility of all reactions is demonstrated in Fig. 4 where

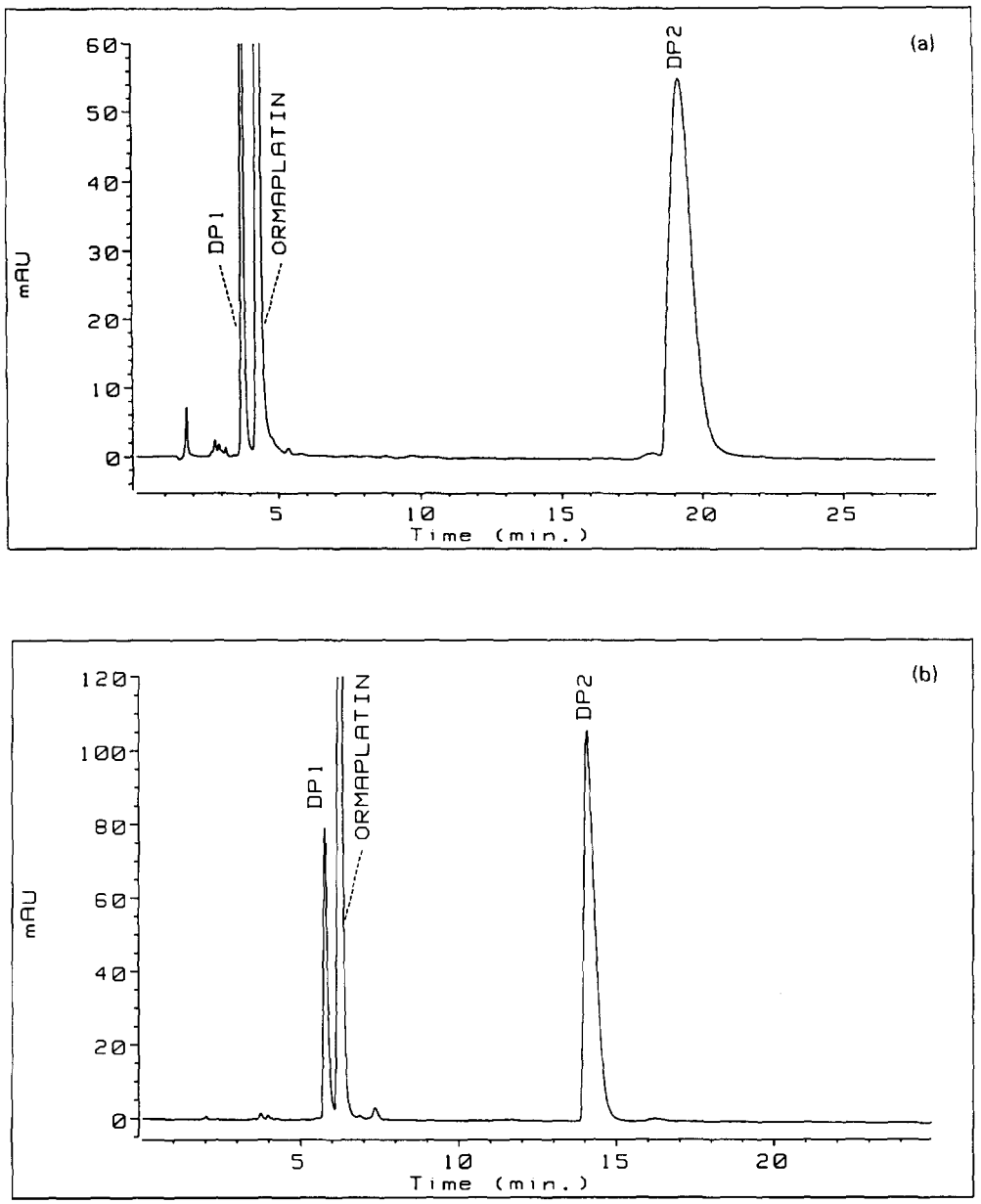

Figure 1
An ormaplatin solution which had been allowed to degrade on the laboratory benchtop for 6 weeks was used to generate chromatograms with the C-18 column (a) and the cyano column (b) using a 100% aqueous mobile phase.

ormaplatin, DP1 and DP2 were initially present at significant levels in 20 mM HCl, but reverted to ormaplatin almost completely within hours.

When DP1 was isolated in pure form (98%) and dissolved in 20 mM HCl (0.5 mg ml^{-1}), only ormaplatin, with very low (<1%) area per cents of DP1 and DP2, was observed by LC after 24 h. If pure DP1 was dissolved in 20 mN H_2SO_4 (0.5 mg ml^{-1}), however, a mixture of three components was observed after 24 h (Fig. 5). Under these conditions the largest response in the chromatogram was associated with DP2. Saline solutions (0.9% NaCl) of DP1 appear to be quite stable over an 8 day time frame, however, with only a small buildup (~5%) of ormaplatin and almost no production of DP2. If DP1 was dissolved in water (0.5 mg ml^{-1}), its concentration decreased steadily with time (being about 25% of its initial level after 8 days) but only small increases in the DP2 or ormaplatin responses

Table 1
Relative retention times observed for ormaplatin, DP1 and DP2 using various stationary phases

HPLC column	Relative retention		
	Ormaplatin	DP1	DP2
PRP-1 (Hamilton)*	1.0	0.41	0.41
C-18 (Alltech)*	1.0	0.78	1.09
C-18 (Waters)†	1.0	0.77	0.87
Cyano (Dynamax)	1.0	0.88	2.25
CS-3 (Dionex)‡	Unretained	0.44	1.0

*Ref. 12 with 100% aqueous mobile phase.
†μ-Bondapack (300 × 4.6 mm, 10 μm, Waters Millipore, Milroy, MA), acetonitrile–water (10:90, v/v).
‡CS-3 analytical column with GS-3 guard column (Dionex Corporation, Sunnyvale, CA) with 10 mM HCl mobile phase. Retention time of DP2 was about 12 min in this system.

(2–3%) were observed at the end of the experiment. Under these circumstances the DP1 was mainly converted, slowly and presumably by an unrelated mechanism, to highly retained materials producing broad chromatographic peaks.

The solution chemistry of DP2 was somewhat different than that observed for DP1 in its dependence on both pH and chloride ion. This is demonstrated in Fig. 6 where a DP2 solution (0.2 mg ml^{-1}) in 20 mM H_2SO_4 was found to be unchanged 4 days after being prepared, but began converting to ormaplatin when NaCl (0.9%) was added. When DP2 was dissolved in saline (0.9%), it converted cleanly to ormaplatin. It is interesting to note that DP2 was not found to produce significant levels of DP1 under any of the conditions studied.

Although the synthetic sample of the dichloro-dihydroxy analogue was not soluble in water to any significant extent, this material was somewhat soluble in 20 mM HCl. The composition of this solution is shown in Fig. 4 as a function of time. These data indicate that ormaplatin, DP1 and DP2 were all present immediately after dissolution, but that ormaplatin was the most stable of these species in the presence of high levels of chloride ion and low pH.

Based on the results reported above, the rapid equilibria occurring in aqueous solutions of ormaplatin are represented on the top half of Fig. 7. DP1 and DP2 both appeared to be in equilibrium with ormaplatin, but the equilibrium between DP1 and ormaplatin seemed to be very sensitive to pH but less sensitive to chloride ion concentration, while the equilibrium between DP2 and ormaplatin appears to be strongly dependent on chloride concentration and less sensitive to pH. Despite the data presented in Fig. 5, where a solution of DP1 at low pH in the absence of Cl^- leads to the formation of both ormaplatin and DP2, there is no evidence that DP1 and DP2 are in direct equilibrium. This is because, at low concentrations of chloride, ormaplatin readily forms DP2. Thus the build up of DP2 starting from pure DP1, under the conditions used to generate the data in Fig. 6, may occur through

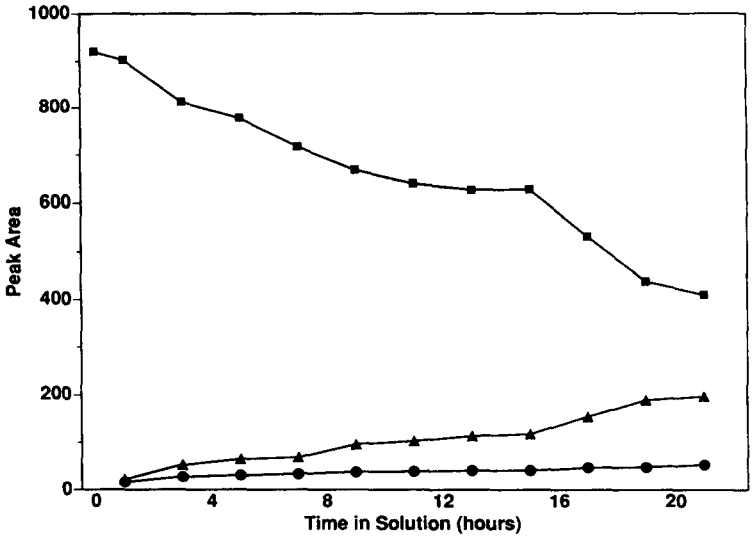

Figure 2
Peak areas of ormaplatin (■), DP1 (●) and DP2 (▲) as a function of time in aqueous solution. Initial ormaplatin concentration was 1 mg ml^{-1}.

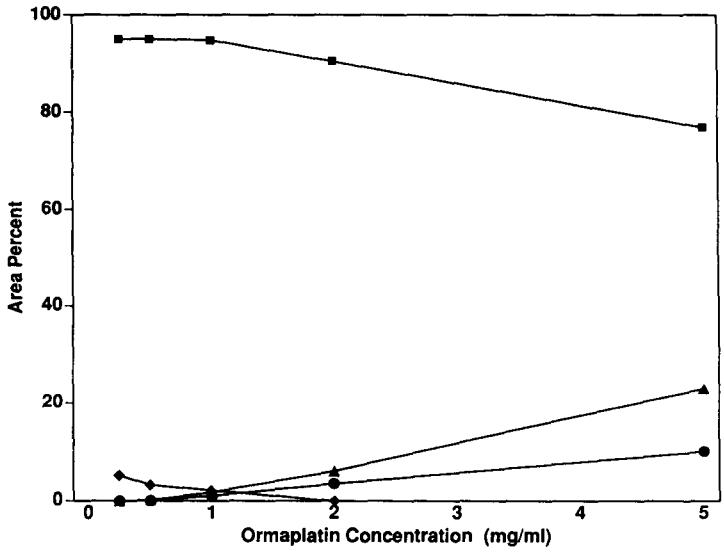

Figure 3
Area per cent composition of aqueous ormaplatin solutions as a function of initial ormaplatin concentration for ormaplatin (■), DP1 (●), DP2 (▲) and platinum-hexachloride (◆). The chromatograms were run immediately after dissolution.

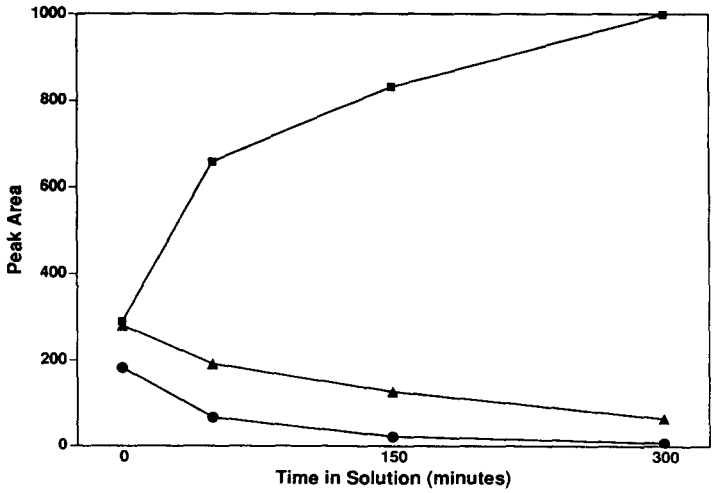

Figure 4
Changes in solution composition as a function of time in a mixture initially containing ormaplatin (■), DP1 (●) and DP2 (▲) at low pH in the presence of excess chloride. The initial solution was prepared by dissolving a synthetic sample of the dichlorodihydroxy analogue of ormaplatin in dilute (20 mM) HCl.

the formation of ormaplatin as an intermediate species.

Slow chemical processes

Although DP1 and DP2 represent the primary decomposition products for the first several hours after ormaplatin has been placed in aqueous solution, at longer times (especially in concentrated solutions) other materials can be observed in the solution chromatograms as later eluting, broad peaks. Once substantial amounts of these later forming decomposition products are generated, a great deal of the reversibility of chemical equilibria associated with chloride ion concentration and pH is lost. At this time little is known about either the solution behaviour or chemistry of these materials, but their late appearance may indicate the need for substantial levels of DP1 and/or DP2 to be present for their formation.

During the process of evaluating whether a final equilibrium composition would be reached by ormaplatin in aqueous solution, another process was observed to take place

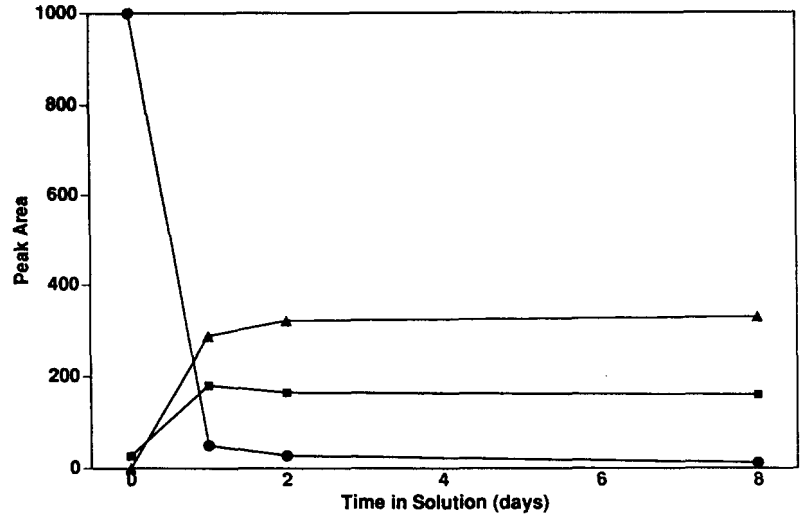

Figure 5
Changes in the composition as a function of time of a solution initially prepared (0.48 mg ml^{-1}) from pure DP1 (●) dissolved in 20 mM sulphuric acid. Also shown are the responses for ormaplatin (■) and DP2 (▲).

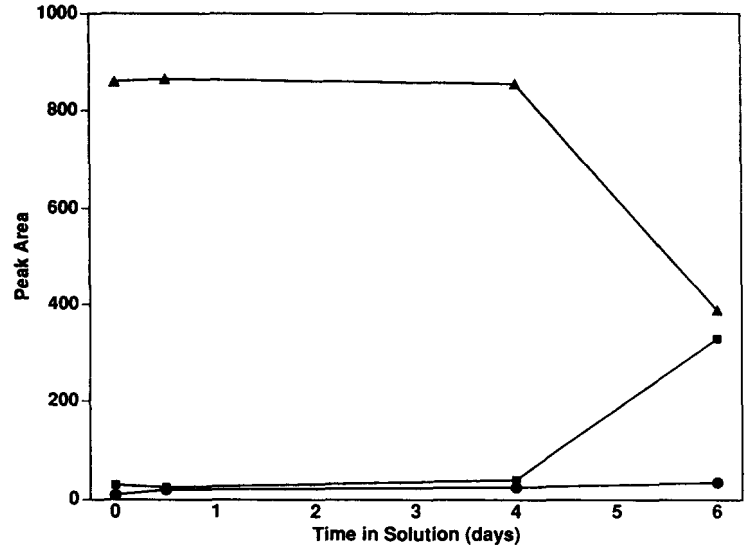

Figure 6
Changes in the composition as a function of time of a solution initially prepared (0.2 mg ml^{-1}) from DP2 (▲) dissolved in 20 mM sulphuric acid. NaCl was added after day 4. Also shown are the responses observed for ormaplatin (■) and DP1 (●).

over the time frame of several days to weeks in all aqueous or saline solutions studied and simply aged in capped vessels on the benchtop. This was the formation of an intensely coloured (orange–brown) precipitate. The chemical composition of these crystals was determined to be: C, 17.34%; H, 3.5%; N, 6.74%; Cl, 26.81% and Pt, 46.6%, which indicates an empirical formula of $C_6H_{14}N_2Cl_3Pt$ and the loss of a chlorine atom from ormaplatin.

The X-ray diffraction data were collected using a clear, thin, rhombic shaped crystal of this orange precipitate with approximate dimensions of 0.15 × 0.15 × 0.015 mm. The structure presented in Fig. 6 (orange solid), in space group $P2_1$, a = 10.580 (5) Å, b = 5.626 (7) Å, c = 9.358 (7) Å, β = 90.06 (8)°, with 1095 reflections in the reduced data set (743 intensities >3σ), was refined to R = 0.038 and R_w = 0.006. Structures of mixed valance platinum complexes with alternating octa-

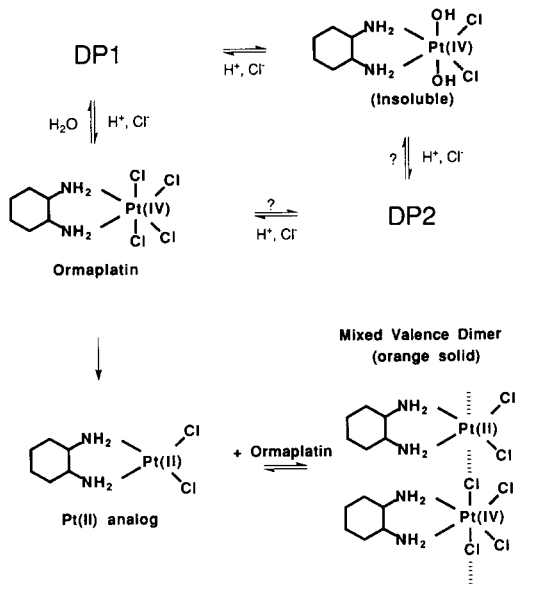

Figure 7
Summary of the solution chemistry observed for ormaplatin in aqueous solution.

hedrally coordinated Pt^{IV} and quadratically coordinated Pt^{II} with bridging chlorine atoms have been reported previously for compounds of this type [18].

When a sample of this orange material was dissolved in water, both ormaplatin and its Pt^{II} analogue were observed chromatographically. This provided additional evidence of the mixed valence nature of this material. The formation of this orange precipitate therefore requires the reduction of Pt^{IV} to Pt^{II}, as indicated in the bottom portion of Fig. 7 followed by the interaction of this reduced material with ormaplatin and subsequent precipitation.

Bulk drug purity

The results of the previous section clearly demonstrate the sensitivity of ormaplatin in aqueous solutions to a variety of factors including initial ormaplatin concentration, pH, Cl^- and light. Assessment of bulk drug purity requires that a solution of the material remain representative of the bulk composition of the material, over the time of analysis, to a degree sufficient to assure the desired level of accuracy. Based on the equilibria observed earlier, sample handling should not include dissolution in HCl or saline solutions, where ormaplatin appears stable, because any DP1 or DP2 that might be present as process impurities would be quickly converted to ormaplatin. The rate at which the conversion of ormaplatin to DP1 and DP2 occurred in water can be diminished to acceptable levels, however, by reducing the initial ormaplatin concentration. At initial ormaplatin concentrations of 0.2 mg ml^{-1} or less significant amounts of DP1 and DP2 were not formed for several hours even in a well-lit laboratory.

When samples of ormaplatin were analysed using concentrations of 0.2 mg ml^{-1} or less, reproducible results were obtained both upon multiple analysis of the same solution or upon analysis of independently prepared solutions. Because of the consistency of this procedure, chromatographic results representative of the composition of the material could be obtained. Chromatographic analysis using dilute solutions of ormaplatin produced an unexpected, early eluting (unretained) peak which was not observed in more concentrated solutions prepared from the same material. This peak was 5–6% of the peak area of ormaplatin and was retained (8–9 min) using an ion exchange column (Dionex AS5). This was the same retention time observed for the synthetic precursor, platinum hexachloride, under these conditions. Ion chromatography was used to evaluate the stability of platinum hexachloride in aqueous solution in the presence of ormaplatin. The results of this determination indicated, for solutions analysed 15 min after mixing a platinum hexachloride solution with solutions containing increasing levels of ormaplatin, the amount of $PtCl_6^{2-}$ found declined steadily with increasing levels of ormaplatin until it was no longer detected at ormaplatin concentrations greater than 2 mg ml^{-1}. When the ormaplatin level was an order of magnitude less (0.2 mg ml^{-1}), however, the level of platinum hexachloride could be accurately determined for over an hour. The effect of ormaplatin concentration on the response observed for platinum hexachloride in freshly prepared aqueous samples is illustrated in Fig. 3.

When samples of ormaplatin or dexormaplatin were dissolved in water, acidic solutions were obtained. For a given lot of material the initial pH observed decreased with increased initial drug concentration, and for a specified drug concentration there was a lot-to-lot variation in the initial pH observed. These pH variations combined with the low levels of Cl^-, which were found when fresh ormaplatin solutions were analysed by ion chromatography,

indicate that HCl is often present in these lots as a process impurity. Because of its involvement in the rapid equilibrium processes, lot-to-lot variations in HCl content can contribute to differences in observed solution behaviour unless the initial concentrations are kept low.

A final process impurity which must be anticipated is the Pt^{II} analogue of ormaplatin with empirical formula $C_6H_{12}N_2PtCl_2$. The formation of the mixed valence dimer in ormaplatin solutions indicates that this material can be formed slowly in solution, but it could also be formed by the presence of Pt^{II} contamination in the starting material. Because of this possibility, a reliable means of resolving this material from ormaplatin is necessary. The C-18 columns tested were not reliable in effecting this separation. Even columns obtained from the same manufacturer would not always provide this resolution and for this reason the cyano-column based chromatography has been developed (Fig. 1).

Conclusions

When ormaplatin is introduced into aqueous solution, a complex series of reactions are initiated. Some of these reactions begin immediately to produce significant levels of platinum containing decomposition products along with a build up in chloride ion concentration and a decrease in pH. Occurring independently, and at a much slower rate under all conditions studied, is the reduction of Pt^{IV} to produce the Pt^{II} analogue which precipitates as the mixed valence dimer when in the presence of excess ormaplatin. Any analytical procedure for the determination of ormaplatin bulk drug purity must adequately control these decomposition processes which are aided by exposure to light.

Because the rate of both the rapid decomposition route of ormaplatin to DP1 and DP2 and the loss of platinum hexachloride from ormaplatin solutions depends strongly on the initial concentration of ormaplatin in solution, samples with adequate solution stability for reproducible and representative analytical determinations, even in the presence of light, can be obtained by preparing samples at a concentration less than 0.2 mg of ormaplatin per ml of water. Analysis of several lots of ormaplatin and dexormaplatin indicated the potential presence of HCl, DP1, DP2, platinum hexachloride and the Pt^{II} analogue of ormaplatin as process impurities. An LC system capable of reliably resolving and responding to all of these materials, except HCl, was developed. Combining this chromatographic system with the relative UV response factors of the various platinum containing species, which can be determined directly by DCP [12] or by atomic absorbance, provides a means of completely characterizing ormaplatin samples.

Although the chemistry of ormaplatin in aqueous solution is understood sufficiently well for the reliable analysis of samples, several interesting questions remain. Additional work is planned to determine the chemical structures of DP1 and DP2, to understand the role of light on the equilibrium involving ormaplatin, DP1 and DP2, and to characterize the mechanism by which platinum hexachloride is lost in ormaplatin solutions.

Acknowledgements — The authors would like to express their appreciation to J.A. Walker, T.A. Runge and P.J. Dobrowolski for numerous helpful discussions and for providing samples of dexormaplatin, the di-hydroxy analogue of ormaplatin and the mixed valance dimer obtained from dexormaplatin.

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[Received for review 29 April 1991; revised manuscript received 9 August 1991]