AN IMPROVED SYNTHETIC PROCEDURE FOR THE PREPARATION OF ^{195m}Pt LABELLED ANTI-TUMOUR COMPLEXES.

H. Jackson[1], C.A. McAuliffe[2]†, A. Perera[1], H.L. Sharma[2] and N.J. Tinker[2].

[1] Department of Medical Biophysics, University of Manchester, Manchester, M13 9PT, UK. [2] Department of Chemistry, University of Manchester Institute of Science and Technology, Manchester, M60 1QD, UK.

SUMMARY

An improved synthetic procedure for the preparation of high specific activity ^{195m}Pt labelled cisplatin, carboplatin and iproplatin is reported. The procedure involves synthetic strategies which optimise the yields at each stage of the synthesis, giving products of high purity and in high yield (ie. cisplatin and carboplatin, 43%, and iproplatin, 22%). A new procedure for the synthesis of carboplatin using silver 1,1-cyclobutanedicarboxylate is also reported.

Key words: Platinum, ^{195m}Pt, anti-tumour, cisplatin, carboplatin, iproplatin

INTRODUCTION

Several analytical techniques have been utilised in order to detect and quantify the distribution and clearance of platinum anti-tumour drugs [1-3]. The methods most commonly used to measure total platinum levels in biological samples are flameless atomic absorption spectrophotometry (F.A.A.S.) and radioisotope labelling [2-4]. Although F.A.A.S. can detect platinum levels as low as 1.0 ng/ml, the procedure involved in sample preparation and data analysis is often time consuming and therefore, disadvantageous when large numbers of samples are involved. The high sensitivity of this method can be matched by the radioactive tracer technique which involves minimum sample preparation.

The radioisotopes of platinum (usually ^{191}Pt and ^{195m}Pt), often used in organ distribution and clearance studies, enable the rapid and accurate measurement of platinum content in a variety of different samples.

Various synthetic procedures have been described for the routine preparation of such labelled complexes [5-7]. The small scale synthesis (involving 8 or 9 steps) necessary for the production of high specific activity, pure material poses its own problems. The full procedure involves modifications of previous

† Author for correspondence

0362–4803/91/101121–10$05.00

Received 25 June, 1991

published synthetic routes overcoming the existing problems of low yield and doubtful purity. In the case of carboplatin the essential procedure is new and a considerable improvement over existing methods.

MATERIALS AND METHODS

All irradiations were carried out at the Harwell isotope production facility, Amersham International P.L.C., Didcot. ^{195m}Platinum was produced by neutron irradiation of enriched ^{194}Pt, purchased from Oak Ridge National Laboratory (ORNL), Oak Ridge, U.S.A.. A typical batch, sealed in a silica vial, used 50 mg of ^{194}Pt (95.06 % enriched) irradiated for 96 h, at a neutron flux of 2.0×10^{14} neutrons s^{-1} cm^{-2}. The specific activity of ^{195m}Pt was approximately 1 mCi/mg Pt. Unless otherwise stated, all reagents used were of Aristar or Analar reagent grade.

At the start of the synthesis the vial was cracked inside a pyrex boiling tube and the platinum sample dissolved in boiling aqua regia. The solution was then transfered to a centrifuge tube (35 ml) in which the rest of the synthesis was carried out (Fig. 1). The amine, A, was ammonia for cisplatin and carboplatin, and isopropylamine for iproplatin. The silver salt of 1,1-cyclobutanedicarboxylate required in the carboplatin synthesis was prepared by adding the stoichiometric quantity of silver nitrate to a near neutralised solution of 1,1-cyclobutanedicarboxylic acid (H_2CBDCA). The white precipitate of Ag_2CBDCA immediately formed was washed with water (10 ml x 3) and dried using acetone and ether. Although the reactants are stoichiometric, because of inevitable losses the course of the synthesis was monitored for ^{195m}Pt and the required quantities of reagents adjusted accordingly.

[^{195m}Pt]Cisplatin

Active platinum metal powder (50 mg) was dissolved in ~7 ml of boiling aqua regia and evaporated to dryness in a water bath using an air-vent. Nitric acid and nitrates were removed by addition of 2.5 ml of concentrated HCl, followed by evaporation to dryness and the procedure repeated 3x, and finally treated with distilled water in the same manner to eliminate HCl.

The residue was now redissolved in 5 ml of distilled water and centrifuged to remove any undissolved material such as silica from the vial which contained the platinum powder. All such radioactive fractions removed were kept in labelled sealed glass vials in order to account for the total platinum present in the reaction tube at any stage of the synthesis. Potassium carbonate (0.33 mmol/ml) was added dropwise until the pH of the solution was 6.5, monitored using pH paper (BDH, range 6-8). The resulting soluble potassium

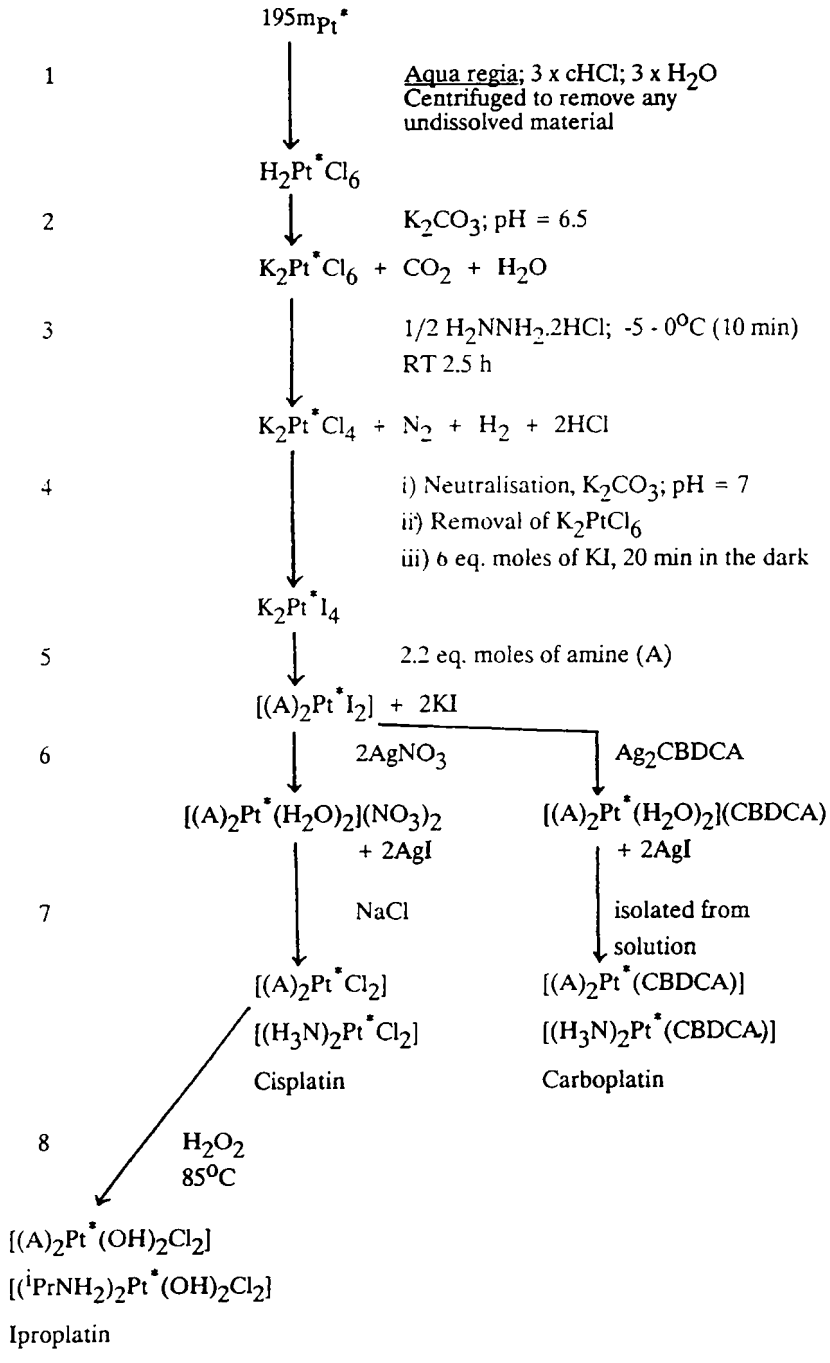

FIGURE CAPTION

Fig.1 The synthetic scheme for preparing [^{195m}Pt] radiolabelled platinum complexes.

hexachloroplatinate was cooled to between -5 and 0 °C and 95% of the stoichiometric quantity of hydrazine hydrochloride (0.1 mmol/ml) added dropwise in ten minutes, and the reaction allowed to proceed at room temperature for 2.5 - 3.0 h.

The resulting acidic solution of potassium tetrachloroplatinate (K_2PtCl_4) was neutralised with potassium carbonate (0.33 mmol/ml) as before and the small amount of unreacted potassium hexachloroplatinate removed by centrifugation. Six molar equivalents of potassium iodide were then added and the reaction left to proceed in the dark for half an hour. To the dark red solution, 2 ml of ammonia solution (~40 μmol/ml) were added dropwise from a 100 μl syringe, and stood 15 minutes. The precipitate of $[Pt(NH_3)_2I_2]$ was separated by centrifugation, the pellet washed with water (3 x 2 ml) and resuspended in 1 ml of water. 75% of the required stoichiometric quantity of silver nitrate was then added and the reaction was left to proceed in the dark for one hour. After centrifugation the supernatant containing the diaquo species, $[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, was transfered to a fresh tube. The pellet was treated with the remaining 25% of $AgNO_3$ solution to complete the reaction. The final pellet of silver iodide was washed with 0.5 ml of distilled water and recentrifuged to extract the residual diaquo compound and added to the main fraction, and repeated if necessary until the activity in the AgI fraction was significantly lower than that of the solution containing the diaquo species.

To the final solution (2 ml) of $[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, 350 mg of NaCl was added with stirring, at room temperature, for two minutes, when the bright yellow precipitate of cisplatin, cis-$[Pt(NH_3)_2Cl_2]$ separated. The product was washed successively with distilled water (2 x 0.2 ml), methanol, ether and air dried.

[^{195m}Pt]Carboplatin

The synthesis of [^{195m}Pt]carboplatin was identical to that of [^{195m}Pt]cisplatin, as far as step 6, Fig. 1. Once the $[Pt(NH_3)_2I_2]$ precipitate was formed it was washed with distilled water (3 x 2 ml) and resuspended in 2 ml of distilled water. Ag_2CBDCA, ~75% of the stoichiometric quantity, was then added and the formation of AgI allowed to proceed in the dark for one hour. After centrifugation, the supernatant was separated and the silver iodide fraction further treated with 20% of the stoichiometric quantity of Ag_2CBDCA in distilled water (1 ml) and left for a further half hour, in order to extract the maximum quantity of activity from the AgI fraction.

The combined supernates were evaporated to less than 1 ml at 40 °C using an air-vent and cold acetone was added to precipitate carboplatin. The product was well washed with acetone and air dried.

[^{195m}Pt]Iproplatin

In the preparation of iproplatin, 40 μmol/ml of isopropylamine, instead of

ammonia. were added in order to precipitate the brown diisopropylaminediiodoplatinum(II) species, $[Pt(^{i}PrNH_2)_2I_2]$. Once the insoluble $[Pt(^{i}PrNH_2)_2Cl_2]$ was formed from this (steps 6 & 7, Fig. 1) the precipitate was washed with distilled water (3 x 10 ml), and resuspended in 7 ml of distilled water. Hydrogen peroxide (0.7 ml of "100 volumes") was added to the solution and the temperature of the reaction mixture raised to 85 ^{o}C. When all the solid had disappeared, the resulting solution was evaporated to ~0.5 ml. Cold acetone was then added slowly to precipitate the iproplatin. The final product was washed three times with acetone, three times with ethylacetate, finally with acetone and air dried.

Quality control

Thin layer chromatography (TLC) was used to determine the purity of the synthesised complexes. Their elution profiles were compared under several elution conditions with those of authentic samples obtained from Johnson Matthey PLC. The elution profiles for almost all the possible impurities were compared with that of the final product, in order to establish the most suitable and efficient solvent system for each compound.

The stationary phase used was alumina glass Merk 60 F_{254} (type E) 200 x 200 x 0.25 mm. The mobile phases are shown in Table 4.

RESULTS

Table 1 shows the product yield and intermediate losses of platinum during a typical microscale synthesis of [^{195m}Pt]cisplatin. Prior to the washing and drying of the product, the overall yield was approximately 57%. Due to the high solubility of cisplatin, approximately 11% of the product was lost when washed with water. Nevertheless a final product yield of ~44% is encouraging for a seven stage microscale synthesis.

Table 2 shows the overall yield of carboplatin in solution to be approximately 47%. After precipitation and washing of the product the final yield was 43%. As with [^{195m}Pt]cisplatin, the percentage yield is promising when the number of stages in the synthesis is considered. The high percentage loss (21%) in the AgI fraction was probably due to incomplete reaction between Ag_2CBDCA and $[Pt(NH_3)_2I_2]$.

The results in Table 3 show the yield in the iproplatin synthesis, up to step 8 (Fig. 1), was approximately 60%. The final yield of iproplatin however, was only 22%. A large percentage loss was found to occur at the final oxidation step of platinum(II) to platinum(IV). It appears that iproplatin is only formed as a minor product and a high percentage of the activity is associated with a deeply coloured, highly acetone soluble impurity.

Quality control of synthesised complexes

The TLC data for [^{195m}Pt]cisplatin is illustrated in Table 4. In all the solvent systems studied the R_f value of the synthesised cisplatin was virtually

Table 1. Final yield of [^{195m}Pt]Cisplatin and intermediate synthesis losses.

Product yield and intermediate losses	Activity in mCi	Percentage activity (of total)
1) Final product cisplatin	16.00	43.75
2) Washings of product (water)	4.15	11.35
3) Washings of product (methanol)	0.62	1.68
4) AgI (silver iodide) fraction	4.75	13.00
5) Residue after formation of $[Pt(NH_3)_2I_2]$	6.97	19.05
6) Residue after formation of K_2PtCl_4	3.75	10.24
7) Residue after formationof H_2PtCl_6	0.34	0.93
Total	36.58	100.00

Table 2. Final [^{195m}Pt]Carboplatin yield and synthesis losses.

Product yield and intermediate losses	Activity in mCi	Percentage activity (of total)
1) Main product carboplatin	13.60	42.78
2) Residue and washings after product formation	1.32	4.15
3) AgI (silver iodide) fraction	6.75	21.23
4) Residue after formation of $[Pt(NH_3)_2I_2]$	6.05	19.04
5) Residue after formation of K_2PtCl_4	3.18	10.00
7) Residue after formation of H_2PtCl_6	0.89	2.80
Total	31.79	100.01

identical to that of the authentic sample. The R_f values of the intermediate complexes were significantly different to those of the final product in the solvent system, acetone:0.1 M HCl (7:3). Table 4 shows the good separation between the possible impurities and the carboplatin in the solvent system acetone:0.1 M HCl (7:3). The R_f values of the final product and the authentic sample were identical under these elution conditions. Due to the reduced migration of carboplatin in the solvent system ethylacetate:acetone:water (45:45:10) it would not be suitable to determine the purity of radiolabelled carboplatin.

The TLC data for labelled iproplatin in different solvent systems is given in Table 4; good separation between the intermediates and final product is observed in all. The crude sample of iproplatin showed at least five, but

Table 3. Final [^{195m}Pt]Iproplatin yield and synthesis losses.

Product yield and intermediate losses	Activity in mCi	Percentage activity (of total)
1) Main product iproplatin	7.07	22.0
2) Residue after formation of product	11.90	36.0
3) Residue during the oxidation of $[Pt(^iPrNH_2)_2Cl_2]$	0.90	3.0
4) Residue after formation of $[Pt(^iPrNH_2)_2Cl_2]$	3.90	12.0
5) Residue in AgI fraction	1.93	6.0
6) Residue after $[Pt(^iPrNH_2)_2I_2]$	5.97	18.0
7) Residue after K_2PtCl_4 and H_2PtCl_6	0.99	3.0
Total	32.66	100.00

Table 4. T.L.C. data (R_f values) for labelled platinum complexes.

	Sovlent system			
	Acetone:0.1 M HCl 7:3	Ethylacetate:Acetone :Water 45:45:10	Acetone:Water 9:1	Isopropanol:Water 7:3
K_2PtCl_6	0.37	0.18	0.39	-
K_2PtCl_4	0.22	0.37	0.24	-
$[Pt(NH_3)_2I_2]$	0.89	0.75	0.82	0.91
Labelled cisplatin	0.63	0.40	0.62	-
Authentic cisplatin	0.65	0.43	0.62	-
Transplatin	0.20	0.88	-	-
Labelled carboplatin	0.59	0.08	-	0.42
Authentic carboplatin	0.58	0.08	-	0.42
$[Pt(^iPrNH_2)_2I_2]$	0.89	0.88	0.91	-
Labelled iproplatin	0.45	0.43	0.42	-
Authentic iproplatin	0.46	0.43	0.43	-

significant, impurity spots when the solvent system ethylacetate:acetone:water 45:45:10 was used. Nevertheless after washing the product thoroughly with acetone (x 3) and ethylacetate (x 3) only one spot was obtained which was of identical R_f value comparred to authentic iproplatin.

Due to the improved conditions used in the synthetic procedure the products were found to be pure (as evident from the TLC data) and did not require any further recrystallization/purification.

DISCUSSION

The optimum volume at the start of the synthesis was found to be 5 ml (±0.5 ml). A large reaction volume resulted in low reaction yields due to incomplete reaction (more likely at low concentrations) and high losses; whereas, a very small reaction volume was expected to give rise to surface effects and therefore uncontrollable reaction rates.

Although other workers have used the sodium salt (Na_2PtCl_6) at the start of the synthesis, this makes it impossible to determine the amount of unreacted Na_2PtCl_6 present after the reduction step, due to the higher solubility of Na_2PtCl_6 under such conditions [6,7], and this in turn perhaps leads to the formation of dark brown iodo-platinum(IV) complexes on adding KI and subsequently platinum(IV) ammoniated products after step 5 [6]. To overcome this problem, the potassium salts were used since K_2PtCl_6 is only partially soluble and at the volumes used (4.5 ml) any unreacted material was present as solid after the reduction and could be removed by centrifugation.

The pH was not allowed to exceed 6.5, to prevent an initial rapid reaction during the addition of the hydrazine.

The reduction was the most crucial step in the synthesis, and hydrazine hydrochloride the most suitable reducing agent. Tin(II) chloride ($SnCl_2$) was not used as reaction between tin(II) and platinum complexes have been reported [8,9]. As hydrazine was found to be a stronger reducing agent than the hydrochloride, the latter was chosen in order to control the reduction process and therefore prevent further reduction of Pt(II) to platinum metal.

Almost all the previously reported synthetic routes used an elevated temperature (85°C) for reduction [5-7]. During initial attempts at the microscale synthesis of cisplatin, it was found that a temperature greater than 80 °C (during step 3) resulted in uncontrolled reduction and hence complete precipitation of platinum metal. This is not surprising as the disproportionation of platinum(II) to platinum(IV) and platinum(0) is known to occur at 60 °C in acid solution, with an equilibrium constant of 22.2 ± 7 M^{-1}, as shown below [10].

$$2\,PtCl_4^{2-} \rightleftharpoons PtCl_6^{2-} + Pt(0) + 2\,Cl^-$$

It has been shown that the reduction of H_2PtCl_6 to H_2PtCl_4 is a slow process and does not rapidly proceed to completion in five minutes at room

period of 2.5-3 h, with 95% of the stoichiometric quantity of $H_2NNH_2.2HCl$, yielded a product free of platinum metal and 90-95 % yield. During the addition of $H_2NNH_2.2HCl$, the temperature of the reaction mixture was kept at a minimum (between -5 to 0 ^oC). Adding the reducing agent at room temperature resulted in the formation of platinum metal to a considerable extent.

Due to the high concentration of HCl present after the reduction with $H_2NNH_2.2HCl$, neutralisation with K_2CO_3 was essential in order to convert any platinum(IV) present as H_2PtCl_6 to K_2PtCl_6.

Addition of six equivalent moles of KI at step 4 was to ensure the complete formation and stabilisation of the partly light sensitive intermediate K_2PtI_4. The use of the iodide species prior to introduction of the ammine (NH_3) is to prevent the formation of a mixture of cis and trans isomers of $[Pt(NH_3)_2Cl_2]$. Owing to the high trans labilising effect of iodide over chloride, the iodide $[PtI_4]^{2-}$ is used at this stage; especially as the trans effect of NH_3 is comparable to that of Cl^- under certain conditions (12).

The rate of addition of ammonia was found to be important during step 5. The rate needed to be relatively slow (20-30 minutes) in order to prevent the possible formation of minute quantities of the trans isomer and/or other platinum impurities (11). Due to various 'geometry effects', the activity in the reaction tube at step 6 was often found to be an inaccurate representation of the mass of platinum present in the precipitate of $[Pt(NH_3)_2I_2]$. The addition of $AgNO_3$ in water, therefore, had to be carried out in stages and the reaction followed carefully, in order to maximise the formation of the diaquo species $[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, but at the same time minimise the reaction volume and contamination by excess silver ions. The percentage loss at this stage was found to decrease when the number of stages of $AgNO_3$ additions was increased. Although silver ions could be effectively removed by precipitation with dilute HCl, using a large excess (10%) of $AgNO_3$ (7) was found to contaminate the final product with silver chloride (AgCl). Attempts to separate completely cisplatin from AgCl resulted in a lower yield of the final product.

Finally, during step 7 the quantity of NaCl added needed to be determined in order to optimise the yield of cisplatin, but also to prevent the presence of excess NaCl.

Carboplatin: An improvement on existing synthetic routes was achieved at step 6 by reacting $[Pt(NH_3)_2I_2]$ and Ag_2CBDCA.

The reaction between the two compounds $[Pt(NH_3)_2I_2]$ and Ag_2CBDCA involved the mixing of two solids. Due to the high solubility of carboplatin in water, the product was obtained by evaporating the final solution to approximately 0.5 ml (at 40 ^oC) and precipitated with acetone. The silver salt of the diacid was used directly in preference to silver nitrate in order to prevent contamination of the product with sodium nitrate. Using the virtually insoluble Ag_2CBDCA meant that contamination by silver ions was minimised. Since any

excess silver ions could not be removed by precipitation with dilute HCl, the addition of Ag_2CBDCA had to be carried out in stages using fractional amounts of the stoichiometric quantity at each stage.

Iproplatin: For the production of iproplatin, the concentration of hydrogen peroxide used in the last stages of the reaction was found to be important. Although at high hydrogen peroxide concentrations the reaction proceeded at a faster rate, this was accompanied by the formation of a deeply coloured highly soluble impurity, the removal of which has proved difficult. The formation of several such contaminants during the final stage of the synthesis has been previously reported [13], but their identity has never been determined. Washing the final product adequately (four to five times) with acetone and ethylacetate was completely effective in their elimination.

ACKNOWLEDGEMENT

The authors wish to thank SERC for funding the PhD of A. Perera and also Johnson Matthey Plc for their generous provision of potassium tetrachloroplatinate(II) salt.

References

1) Riley C.M. and Sternson L.A. - Pharm. Int., 15-19 (1984).
2) Pera M.F. and Harder H.C. - Clin. Chem., 23, 7, 1245-1249, (1977).
3) Vermorken J.B., Van der Vijgh W.J., Klein I., Gall H.E. and Pinedo H.M. - Eur. J. Cancer Clin. Oncol., 18, 11, 1069-1074, (1982).
4) De Simone P.A., Yancey R.S., Coupal J.J., Butts J.D. and Hoeschele J.D. - Cancer Treat. Rep., 63, 6, 951-960, (1979).
5) Wolf W., Manaka R.C. and Ingalis R.B. In: 'Radiopharmaceuticals and labelled compounds', International Atomic Energy Agency, Vienna, Vol (II), pp 205, (1973).
6) Hoeschele J.D., T.A. Butler, Roberts J.A. and Guyer C.E. - Radiochim. Acta, 31, 27-36, (1982).
7) Baer J., Harrison R., McAuliffe C., Zaki A., Sharma H. and Smith A. - Int. J. Appl. Radiat. Isot., 36, 3, 181-184, (1985).
8) Gile J.D., Garrison W.M. and Hamilton J.G. - J. Chem. Phys., 19, 1426, (1951).
9) Young J.F. - Adv. Inorg. Chem. Radiochem., 11, 117-121, (1968).
10) Ginstrup O. and Leden I. - Acta Chem. Scand., 21, 2689-2694, (1967).
11) Hobson C.. M.Sc. Thesis, University of Manchester, (1985).
12) Hartley F.R. - In: 'The Chemistry of Platinum and Palladium'. Applied Science Publishers Ltd., England, (1973).
13) Hoeschele J.D., Ferren L.A., Roberts J.A. and Whitfield L.R. - In: 'Platinum coordination complexes in cancer chemotherapy' (Eds. M.P. Hacker, E.B. Douple and I.H. Krakoff), Martinus Nijhoff Publishing, Boston, USA, pp 103-113, (1984).