An Alternative HPLC Method for Analysing Mixtures of Isomeric Platinum(II) Diamine Compounds

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The anticancer activity of divalent platinum compounds of the type $Pt(A)_2X_2$ (where A = ammonia or amine and X = a monovalent anion) usually correlates with the geometry of the compound, the *cis*-isomer being active while the corresponding *trans*-compound is inactive [1]. Synthesis of these compounds, however, often results in mixtures of *cis*- and *trans*-isomers. UV spectroscopy is widely employed to determine the purity of an unknown sample, although minor impurities resulting from the other isomer are extremely difficult to detect by this method. A sensitive and widely applicable method for analysing both of the two isomers simultaneously would therefore be of some importance, especially when these compounds are used as drugs. The UV spectroscopic procedure reported for the analysis of mixtures of *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ requires the characterization of reaction products, the isolation of which may be difficult in the case of other Pt(II) diamine compounds, thus limiting its applicability [2]†.

Traditionally the determination of the geometry of an unknown Pt(II) diamine compound is based on Kurnakow's test, which involves the addition of thiourea (tu) to the aqueous suspension of the sample. On gentle heating, *trans*-$Pt(A)_2X_2$ gives *trans*-$[Pt(tu)_2(A)_2]X_2$ which is colourless, while the *cis*-isomer produces yellow $[Pt(tu)_4]X_2$ [3]. As an analysis method it is rather cumbersome and insensitive, because it involves the selective precipitation and weighing of the thiourea complexes. Instead, the use of HPLC gives a convenient and more sensitive way for analysing the thiourea complexes. Woollins *et al.* [4] have used this method for the detection of trace amounts of *trans*-$Pt(NH_3)_2Cl_2$ in the presence of *cis*-$Pt(NH_3)_2Cl_2$ using a strong cation exchange column. Here we wish to report that mixtures of isomeric $Pt(A)_2Cl_2$ (where A = NH_3, NH_2CH_3 or $NH(CH_3)_2$) can be analysed with sufficient accuracy and reproducibility after thiourea treatment using a reversed-phase column.

†We note that according to our findings (ref. 6) the ϵ (300 nm) for *cis*-$Pt(NH_3)_2Cl_2$ is higher by about 10%.

Experimental

K_2PtCl_4, purchased from Degussa, was used as received. Thiourea, analytical grade from Riedel-De-Haen, was crystallized from methanol. The *cis*- and *trans*-isomers of $Pt(NH_3)_2Cl_2$, $Pt(NH_2CH_3)_2Cl_2$ and $Pt(NH(CH_3)_2)_2Cl_2$ were prepared by known methods [5]. *cis*-$Pt(NH_3)_2Cl_2$ was purified by DMF crystallization [6], while the other *cis*-products were treated with acetone to remove any traces of the *trans*-isomer, after which they were recrystallized from water. The corresponding *trans*-compounds were purified by repeated recrystallizations from water. The thiourea complexes, *trans*-$[Pt(tu)_2(A)_2]Cl_2$ (where A = NH_3, NH_2CH_3 or $NH(CH_3)_2$), were prepared by treating the corresponding *trans*-diamine compounds with thiourea [3]. $[Pt(tu)_4]Cl_2$ was synthesized analogously from K_2PtCl_4. The elemental compositions found for each of the prepared compounds correlated well with the calculated values.

HPLC measurements were performed by employing a system consisting of a Perkin-Elmer Series 1 LC-pump, an RP-18 column (Techopak 10C18, 300 X 3.9, HPLC-Technology, U K.) and a Perkin-Elmer LC-75 spectrophotometric detector working at 260 nm. Isocratic elution with a flow rate of 1.2 cm^3 min^{-1} was used throughout and the peak height was taken as a measure of the concentration. The most suitable eluent for optimal separation of thiourea, *trans*-$[Pt(tu)_2(A)_2]Cl_2$ and $[Pt(tu)_4]Cl_2$ was found to depend drastically on the nature of the ligand A. When A is ammonia the best resolution was achieved by using 0.1 mol dm^{-3} NH_4Ac in H_2O/MeOH (95/5) as an eluent (eluent 1). In the case of methyl substituted amines, *viz.* NH_2CH_3 and $NH(CH_3)_2$, eluents 2 and 3, respectively, gave improved resolution*. Unfortunately neither of these eluents is suitable when A = NH_3, because the signals for thiourea and *trans*-$[Pt(tu)_2(NH_3)_2]Cl_2$ are not resolved. All the eluents were thoroughly degassed by sonification under reduced pressure before use.

The analytical procedure employed is as follows. To a known amount of $Pt(A)_2X_2$ (b mmol), dilute HNO_3 (2b mmol) and thiourea (10b mmol) were added**. The reaction mixture was allowed to stand in a water bath for 200 min at 40 °C (or overnight at room temperature) with occasional

*Eluent 2: 5×10^{-4} mol dm^{-3} HNO_3 in 5×10^{-2} mol dm^{-3} $NaClO_4$. Eluent 3: 5×10^{-4} mol dm^{-3} HNO_3 and 5×10^{-2} mol dm^{-3} $NaClO_4$ in H_2O/MeOH (95/5).

**Total Pt(II) concentration should not exceed 0.01 mol dm^{-3} at this stage.

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shaking. After cooling to room temperature, samples (diluted with water when necessary) were analysed by HPLC using the suitable eluent. In all cases the signal height was found to correlate linearly with concentration in a logarithmic scale in the concentration range from 5×10^{-6} to 2×10^{-3} mol dm^{-3}. Standards for the analytical procedure were prepared using pure samples of *trans*-$[Pt(A)_2(tu)_2]Cl_2$ and $[Pt(tu)_4]Cl_2$ dissolved in water at known concentrations.

Results and Discussion

Several samples of *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ were treated with thiourea as described above. The subsequent chromatographic analysis of each reaction mixture revealed only one product, which had a retention time of 3.8 min in the case of *trans*-$Pt(NH_3)_2Cl_2$, while that from the *cis*-isomer appeared at 8.0 min. These retention times are identical with those of *trans*-$[Pt(tu)_2(NH_3)_2]Cl_2$ and $[Pt(tu)_4]Cl_2$, respectively, observed from authentic samples. With eluent 1 the free thiourea has a retention time of 2.8 min. The dissolution of the parent compounds in water before thiourea treatment has no effect on the reaction products. Samples prepared in this way gave identical results to those in the solid state. In all cases yields corresponding to 96–100% of the theoretical amounts were observed. It is noteworthy that DMF crystallization [6] seems to yield 'pure' *cis*-$P(NH_3)_2Cl_2$, *i.e.* a product without detectable amounts of *trans*-isomer. However, a commercial sample of *cis*-$Pt(NH_3)_2Cl_2$ tested was found to contain about 11% of the *trans*-isomer.

Stock solutions from *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ were combined to yield various sets of mixtures in which the total Pt(II) concentration remained constant. Subsequent analysis of these mixtures revealed that the method works satisfactorily, as demonstrated in Fig. 1A. In all cases the difference between the actual and found concentrations was smaller than 3%. Reaction mixtures containing minor amounts of the other isomer in the presence of a large and constant excess of one isomer were used to study the sensitivity of the method. Figure 2 shows that in both cases the presence of the other isomer as an impurity can quantitatively be determined at the level of about 0.5%. At the same time the method allows quantification of the main component. At concentrations below 5×10^{-6} mol dm^{-3} the signal for *trans*-$[Pt(tu)_2(NH_3)_2]Cl_2$ is partly hidden by the tailing of the free thiourea signal, thus producing a larger error for the determination of the *trans*-isomer. The same lower limit holds also for the analysis of *cis*-$Pt(NH_3)_2Cl_2$ in the presence of large amounts of the *trans*-isomer. The slow reaction of *trans*-$[Pt(tu)_2(NH_3)_2]Cl_2$ with excess thiourea gives small amounts of $[Pt(tu)_4]Cl_2$, thus producing an error for the determination of the *cis*-isomer at lower concentrations*. The upper limit of 2×10^{-3} mol dm^{-3} for the analysis method is merely determined by the limitations of our chromatographic system. At higher concentrations the signal shape of both reaction products was found to change, resulting in a non-linear correlation between the signal height and concentration.

*The actual reaction time depends on the nature of the ligands A and X. When X = Cl^- ion, the reactions are complete within 200 min with A = NH_3, and within 300 min with A = NH_2CH_3 or $NH(CH_3)_2$, without any detectable degradation of either product. With slower exchangeable ligands, e.g. Br^- and I^- ions, longer reaction times may be needed. Prolonged standing at 40 °C may, however, cause the conversion of *trans*-$[Pt(tu)_2(A)_2]X_2$ to $[Pt(tu)_4]X_2$. For example, the reaction *trans*-$[Pt(tu)_2(NH_3)_2]Cl_2 + 2tu = [Pt(tu)_4]Cl_2 + 2NH_3$ has a half-live of about 10 days at 40 °C in 0.1 mol dm^{-3} thiourea solution.

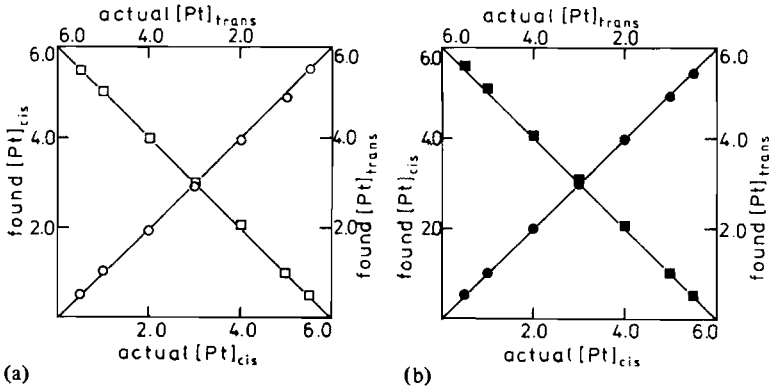

Fig. 1. Test analysis of mixtures of (a) *cis*(○)- and *trans*(□)-$Pt(NH_3)_2Cl_2$ and (b) *cis*(●)- and *trans*(■)-$Pt(NH(CH_3)_2)_2Cl_2$ after thiourea treatment at constant Pt(II) total concentration (6×10^{-4} mol dm^{-3}).

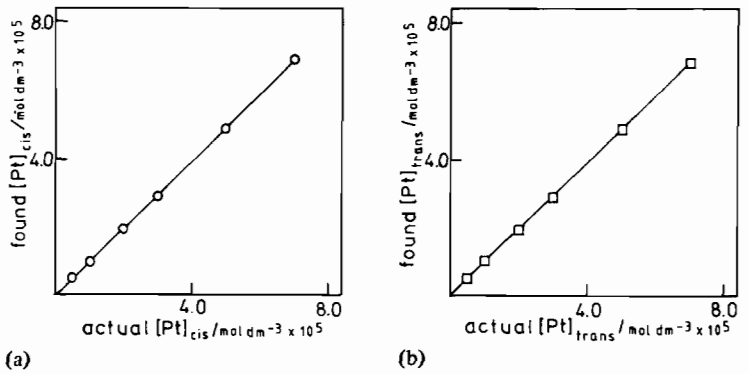

Fig. 2. Detection of *cis*-$Pt(NH_3)_2Cl_2$ (○) as an impurity in the presence of the *trans*-isomer (9×10^{-4} mol dm^{-3}). (b) Detection of *trans*-$Pt(NH_3)_2Cl_2$ (□) in the presence of the *cis*-isomer (1×10^{-3} mol dm^{-3}).

The analysis method reported can also be applied for other Pt(II) diamine compounds. The treatment of *cis*- and *trans*-$Pt(A)_2Cl_2$ (where $A = NH_2CH_3$ or $NH(CH_3)_2$) with thiourea yields in all cases only one detectable product. With NH_2CH_3 compounds the product from the *trans*-isomer has a retention time of 5.0 min, while that from *cis*-isomer appears at 8.0 min using eluent 2. In the case of $NH(CH_3)_2$ compounds the corresponding products appear at 7.8 and 5.0 min, respectively, using eluent 3. These retention times agree with those from the authentic samples, *viz.* *trans*-$[Pt(tu)_2(A)_2]Cl_2$ and $[Pt(tu)_4]Cl_2$. With both eluents the free thiourea appears at about 2.8 min. Figure 1B shows that a good correlation exists between the actual and found values in the case of $Pt(NH(CH_3)_2Cl_2$ complexes. For both *trans*-$Pt(A)_2Cl_2$ compounds the lower analytical limit is below 5×10^{-6} mol dm^{-3} because of more favourable elution conditions.

It was subsequently found that the compound $[Pt(NH_3)_3Cl]Cl$ also reacts with thiourea to give *trans*-$[Pt(tu)_2(NH_3)_2]Cl_2$ as a sole product. Therefore additional methods are required to ascertain the source of *trans*-$Pt(tu)_2(A)_2X_2$ when this method is applied in practice.

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