# A Spectrophotometric Method for analysing Mixtures of cis- and trans-Diamminedichloroplatinum(II) in Solution

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Summary Solutions containing the anti-cancer agent cis-[Pt(NH$_3$)$_2$Cl$_2$] and its inactive trans isomer can be quantitatively analysed by measuring optical absorbances before and after controlled reaction with allyl alcohol.

cis-[Pt(NH$_3$)$_2$Cl$_2$] is a potent anti-tumour drug.[1] Under certain conditions it can isomerise to the more stable and inactive trans form.[2,3] The problem of analysing mixtures of the two isomers in aqueous solution is therefore of some importance. The only known quantitative method is the cumbersome one of Kurnakow[3,4] which involves addition of thiourea and the selective precipitation and weighing of the thiourea complexes. No satisfactory instrumental method exists: paper chromatography,[5] t.l.c.,[6] and h.p.l.c. are employed mainly for detecting one isomer in the presence of the other, Pt n.m.r. spectroscopy proved of insufficient sensitivity at low concentrations, and laser Raman spectroscopy did not yield useful signals.

Spectrophotometric analysis would be ideal since visible/ u.v. instruments are widely available. However, the optical spectra differ significantly only at 300 nm where the extinction coefficients $\epsilon$ for the cis and trans forms are 118 and 54 dm$^3$ mol$^{-1}$ cm$^{-1}$, respectively. A search was therefore made for a reagent to transform the isomers into new complexes possessing quite different spectra. Of the many reagents tried, only nitrite and allyl alcohol (AA) showed promise. Both reacted much faster with the trans than with the cis isomer. When times were chosen at which the trans form had almost completely reacted, the solution spectra differed markedly. The absorbances at suitable wavelengths (270 nm for nitrite, 252 nm for AA) of the trans solution were then an order of magnitude greater than those of an equimolar cis solution. Kinetic studies subsequently showed that the rate of the trans substitution reaction was not sufficiently reproducible with nitrite (even in buffered solutions) but quite reproducible with allyl alcohol. The reaction is first order in AA and in trans isomer.[7] The analytical procedure with AA is set out below.

Let the solution in question contain cis (of concentration $c_{cis}$ mol dm$^{-3}$) and trans (of concentration $c_{tr}$ mol dm$^{-3}$). First, measure the absorbance ($A_u$) at 300 nm at room temperature in a cell of length $l$ cm (equation 1). Next,

$$A_u/l = 118c_{cis} + 54c_{tr} \qquad (1)$$

dilute a portion of the solution with an equal volume of AA solution (0·06 mol dm$^{-3}$; 1 cm$^3$ AA in 250 cm$^3$ solution) and allow the mixture to stand for 100 min at 40 °C (or for 6 h at 25 °C). This converts all the trans form into[7,8] [Pt(NH$_3$)$_2$Cl AA]$^+$ with $\epsilon$ 2030 dm$^3$ mol$^{-1}$ cm$^{-1}$ at 252 nm. At this stage the cis isomer, converted to a small extent only, exhibits an effective extinction coefficient of 237 (or 202 at 25 °C). Measure the absorbance of the mixture ($A_s$) at 252 nm at room temperature, with 0·03 mol dm$^{-3}$ AA in the reference cell (equation 2). Solution of these two

$$A_s/l = 118\cdot5c_{cis} + 1015c_{tr} \qquad (2)$$

simultaneous equations leads to equations 3 and 4 if the AA reaction was carried out at 40 °C.

$$c_{cis} = 8\cdot82 \times 10^{-6}(1015A_u - 54A_s)/l \qquad (3)$$

$$c_{tr} = 8\cdot82 \times 10^{-6}(118 A_s - 118\cdot5 A_u)/l \qquad (4)$$

Various sets of mixed cis and trans isomer were prepared in 0·1 mol dm$^{-3}$ KCl solution to suppress hydrolysis to the aquo-forms;[9] this concentration of Cl$^-$ is biologically significant since it is the same as that found in plasma.[10] They were then analysed as described. The Figure demonstrates that the method works satisfactorily. Excluding
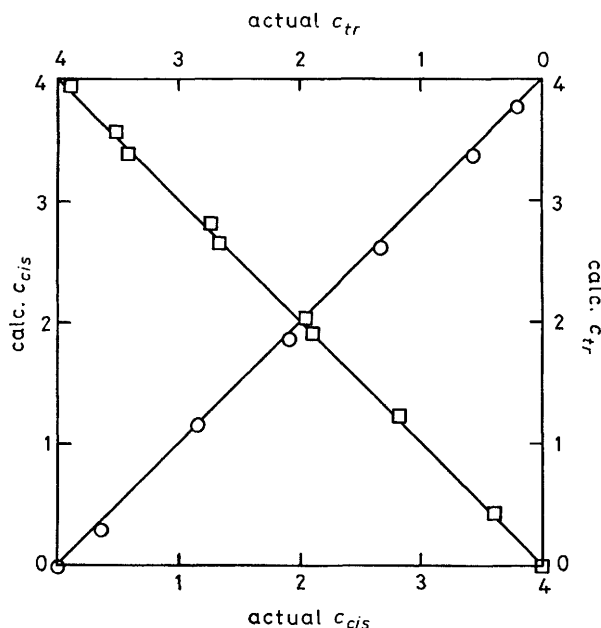


FIGURE. Tests of the analytical procedure using the allyl alcohol reaction at 40 °C for the cis isomer (○) and the trans isomer (□). Vertical axis left: calc. $c_{cis}$/10$^{-4}$ mol dm$^{-3}$; vertical axis right: calc. $c_{tr}$/10$^{-4}$ mol dm$^{-3}$; horizontal axis top: actual $c_{tr}$/10$^{-4}$ mol dm$^{-3}$; horizontal axis bottom: actual $c_{cis}$/10$^{-4}$ mol dm$^{-3}$.

molarities below 4 × 10$^{-5}$ mol dm$^{-3}$ where small absolute errors produce large relative ones, the mean difference between the calculated and actual concentrations of the cis form was 1·3% (2·4% at 25 °C) and of the trans form 2·1% (2·1% at 25 °C). Although the AA reaction would be faster at a still higher temperature, we do not recommend work much above 40 °C because the substitution kinetics change around 50 °C.[7]

Several cis and trans solutions of known concentration were also prepared without KCl, and allowed to stand for several days to reach hydrolysis equilibrium. Most of the cis isomer will then have been in the monoaquo-form.[9] At

this point sufficient KCl was added to make the solution $0.1$ mol dm$^{-3}$, and the system was allowed to re-equilibrate for 1 h. The accepted *cis* and *trans* absorption spectra then reappeared, and application of the normal analytical procedure again led to concentrations in satisfactory agreement with the actual values.

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