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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF POTENTIAL ANTITUMOR PLATINUM(II) COMPLEXES OF 1R,2R-CYCLOHEXANEDIAMINE

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High performance liquid chromatography of antitumor Pt(II) complexes of 1R,2R-cyclohexandiamine, as well as cis- and trans-DDP, dichlorodiammineplatinum(II), has been performed, using a Toyo Soda G1000PW column, eluting with 0.1 mol dm^{-3} Na_2SO_4, and various Pt(II) complexes were separated successfully.

The authors prepared highly antitumor active water-soluble mono- and bis-D-glucuronato Pt(II) complexes of 1R,2R-cyclohexanediamine(=dach).[1] They are so soluble in water that the isolation of those water-soluble Pt(II) complexes was not easy. But the separation among Pt(D-glucuroanto)(NO_3)(dach)(=mono-glucu), Pt(D-glucuronato)$_2$(dach)(=bis-glucu), and Pt$(NO_3)_2$(dach) has been finally achieved by means of column chromatography adopting a Sephadex G10 column eluted with water. After reacting Pt$(NO_3)_2$(dach) with sodium D-glucuronate in the ratio of 1:1 for 3 weeks at room temperature, the solution was separated through a Sephadex G10 column with water as an eluent. The first band eluted contained mainly bis-glucu, and the second and the third bands corresponded to mono-glucu and dinitrato complexes, respectively.

I. Synthesis of Pt(D-glucuronato)$_2$(dach): Two grams of Pt$(NO_3)_2$(dach) H_2O was dissolved in H_2O(40 cm^3) by warming on a steam bath. The solution was cooled to room temperature and was added 2.5 g of sodium D-glucuronate dissolved in H_2O (5 cm^3). The resultant solution was covered with aluminum foil and kept standing for 2 weeks at room temperature. It was concentrated under reduced pressure to 10 cm^3 at ca. 40°C. The concentrate was chromatographed through a Sephadex G10 column (26 ID x 1000 mm) with H_2O and the objective complex was eluted in the 1st main band. (Anal., Calcd. for $PtC_{18}H_{36}O_{14}N_2$: C; 29.53, H; 4.97, N; 3.83 %. Found: C; 29.72, H; 4.91, N; 4.02 %)

II. Synthesis of Pt(NO_3)(D-glucuronato)(dach): Mono-glucu was also obtained in the same method of preparing bis-glucu, except mixing Pt$(NO_3)_2$(dach)$\cdot H_2O$(2 g) and sodium D-glucuronate(1.2 g) in the ratio of 1:1. The objective complex was eluted in the 2nd band through the same column. (Anal., Calcd. for $PtC_{12}H_{23}O_{12}N_3$: C; 24.00, H; 4.53, N; 7.00 %. Found: C; 24.10, H; 4.35, N; 6.68 %)

III. Synthesis of PtCl(D-glucuronato)(dach): Two grams of bis-glucu dihydrate was dissolved in H_2O(12 cm^3) and 0.21 g of KCl was dissolved in H_2O (3 cm^3). Both solutions were cooled to 5°C and mixed. The resultant solution was

left in a refrigerator for 16 hr. The yellow precipitate was filtered off and the filtrate was passed through a column containing 2 cm^3 of Amberite IR-120 and IRA-400 each in order to remove the excess KCl. The chromatographic separation using a Sephadex G10 column was carried out in a cold chamber (5°C). The objective complex was eluted in the 2nd band. (Anal., Calcd. for $PtC_{27}H_{25}N_2Cl$: C; 25.92, H; 4.54, N; 5.04 %. Found: C; 25.18, H; 4.41, N; 4.97 %)

A high performance liquid chromatograph(Shimazu LC-3A) was equipped with a gel permeation column(TSK Gel PW1000, 7.5 ID x 600 mm) connected to its pre-column (7.5 ID x 50 mm) and a UV detector(Shimazu SPD-2A). One tenth mol dm^{-3} Na_2SO_4 solution was used as an eluating solvent (flow rate 1.0 cm^3/min).

There are some papers[2-6] of high performance liquid chromatography, HPLC study of DDP[2,3] and other Pt complexes[4-6], using ion exchange columns, but the coordination of the sugar carboxylates to the central platinum is not so strong that an use of such ion exchange columns is not suitable.

We recently tried to use a TSK gel PW1000 column and found it very useful in the separation of dach Pt(II) complexes. In this paper, we describe the HPLC conditions to separate various antitumor Pt(II) complexes of dach isomer, as well as cis- and trans-DDP. We examined various eluates, i. e., H_2O, 0.2 mol dm^{-3} Na_2HPO_4, 0.1 mol dm^{-3} $NaNO_3$, 0.025 mol dm^{-3}, 0.05 mol dm^{-3}, 0.1 mol dm^{-3}, and 0.2 mol dm^{-3} Na_2SO_4. Water did not separate bis-glucu and mono-glucu complexes. Two tenths mol dm^{-3} Na_2HPO_4 and 0.1 mol dm^{-3} $NaNO_3$ eluates separated bis- and mono-glucu complexes, but they could not differenciate the latter complex from aqua species. So we examined Na_2SO_4 solutions as an eluate, changing the concentrations and 0.1 mol dm^{-3} gave the best separation among the eluates examined as shown in Table I.

Table I HPLC Separation of Pt(II) Complexes of Dach with Various Eluates

Eluates \ Complexes	bis-glucu* t_R (min)	mono-glucu* t_R (min)	$(NO_3)_2$ t_R (min)	mono-Cl** t_R (min)	Cl_2 t_R (min)
0.2 mol dm^{-3} Na_2HPO_4	18.6	21.8	21.8	--	39.5
0.1 mol dm^{-3} $NaNO_3$	20.3	25.0	25.0	--	46.6
H_2O	15.2	15.2	22.0	--	--
0.025 mol dm^{-3} Na_2SO_4	18.0	21.0	22.4	25.6	38.0
0.05 mol dm^{-3} Na_2SO_4	18.2	21.1	22.4	25.6	39.0
0.1 mol dm^{-3} Na_2SO_4	18.4	21.4	23.6	26.6	40.0
0.2 mol dm^{-3} Na_2SO_4	19.0	21.6	24.0	27.0	43.0

* Bis- and mono-glucu stand for Pt(D-glucuronato)$_2$(dach) and Pt(D-glucuronato)(NO_3)(dach), respectively.

**Mono-Cl stands for PtCl(D-glucuronato)(dach).

All sample solutions were prepared by dissolving 4 mg of samples in H_2O (1 cm^3).

Table II HPLC Retention Times of Pt(II) Complexes of Dach

Complexes	t_R (min)	Complexes	t_R (min)
Pt(D-glucuronato)$_2$(dach)	18.4	$PtCl_2$(dach)*	40.0
Pt(NO_3)(D-glucuronato)(dach)	21.4	$PtBr_2$(dach)	44.8
Pt(NO_3)$_2$(dach)	23.6	PtI_2(dach)	--
Pt(SO_4)(dach)	23.6	Pt(oxalto)(dach)	62.8
PtCl(D-glucuronato)(dach)	26.6	Pt(malonato)(dach)	36.6
PtBr(D-glucuronato)(dach)	30.4	cis-$PtCl_2(NH_3)_2$*	95.2
PtI(D-glucuronato)(dach)	40.0	trans-$PtCl_2(NH_3)_2$*	66.0

*These samples were saturated in 0.1 mol dm^{-3} NaCl solutions.

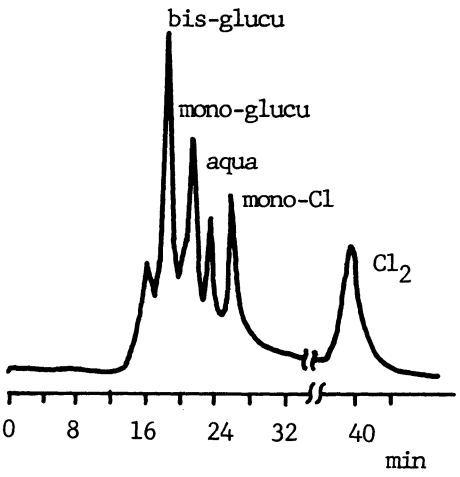

Fig. 1 A chromatogram illustrates separation among D-glucuronato Pt(II) complexes.

With adopting 0.1 mol dm^{-3} Na_2SO_4 as an eluate, various Pt(II) complexes of dach were separated relatively well as shown in Fig. 1 and Table II.
A typical chromatogram illustrating the separation among D-glucuronato Pt(II) complexes is shown in Fig. 1.
Pt(NO_3)$_2$(dach) and Pt(SO_4)(dach) showed the same retention time by changing concentrations of Na_2SO_4 and did not show any change in their chromatographic behavior, suggesting the existence of the aqua species.

A chromatogram illustrated in Fig. 2A was one which was taken immediately after dissolution of bis-glucu complex in water and a main peak was observed at the solvent front(t_R=16.0 min) with an additional peak at t_R=18.4 min. In 2 hr, the main peak decreased, followed by the increment of two peaks at t_R=18.4 and 21.4 min. With 24 hr standing, bis-glucu showed a chromatogram with a main peak at t_R=18.4 min as shown in Fig. 2C, which showed little change afterward. The similar chromatographic behavior was also observed for mono-glucu complex, except that the peak at t_R=21.4 min became larger in 2 hr, then the peak at t_R=18.4 min became gradually larger, suggesting the formation of bis-glucu complex.

With the additon of excess sodium D-glucuronate to this mono-glucu solution, the peak at t_R=18.4 increased, followed by the decrease of the peak heights at t_R= 21.4 and 23.6 min, the latter of which is assignable to the aqua species. These facts suggest that the peaks at t_R=18.4 and 21.4 min are assignable to bis-glucu and mono-glucu complexes, respectively. The chromatographic behavior indicates the following equilibrium exists for bis- and mono-glucu complexes.

Separation among D-glucuronato Pt(II) complexes and aqua species agrees with the order of molecular weights, so that the peak observed at the solvent front

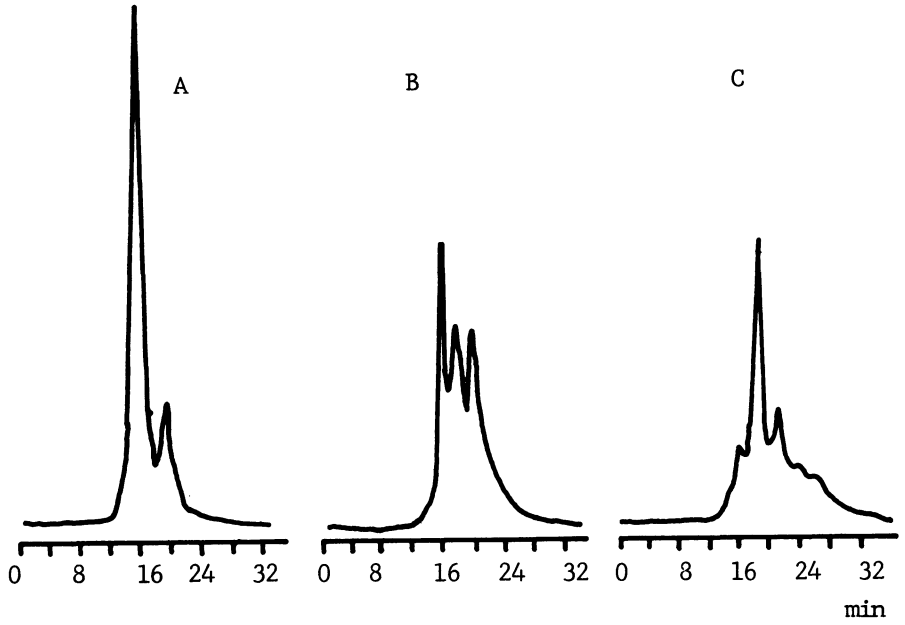

Fig. 2 Time dependence of chromatograms of bis-glucu complex
A: immediately after dissolution B: after 2hr C: after 24 hr

$$2\ Pt(D\text{-glucuronato})_2(dach) \rightleftharpoons Pt(D\text{-glucuronato})(H_2O)(dach) + D\text{-glucuronate}$$

$$2\ Pt(D\text{-glucuronato})(H_2O)(dach) \rightleftharpoons Pt(D\text{-glcuronato})_2(dach) + Pt(H_2O)_2(dach)$$

seems to indicate the existence of the aggregated form with somewhat larger molecular weight. The gel PW1000 was also applied to identification of cis- and trans-DDP. The aqueous solution containing cis-DDP gave a relatively complicated chromatogram with several peaks, which change their peak heights with the elapse of time due to the formation of hydrolysis products. In 0.1 mol dm^{-3} NaCl solution, each of cis- and trans-DDP gave only one peak at t_R=95.6 and 66.0 min, respectivley. More detailed study is now under investigation and will be reported in near futrue.

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