

SYNTHESIS AND CHARACTERIZATION OF POLYMER DERIVATIVES OF *cis*-PLATINUM COMPLEXES*

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ABSTRACT

Cis-platinum complexes used as antitumor drugs were attached onto preformed polymers to reduce the toxicity. Either alternating copolymer of styrene and maleic anhydride (CPSMA) or polyacrylic acid (PAA) is readily dissolved in alkaline solution. The *cis*-platinum complexes are easily converted into water soluble species when treated by silver nitrate in water. The expected products could be obtained when the two aqueous solutions were brought together. The products were characterized by elementary analysis, IR and XPS. The polymer derivatives could exchange ligands with nucleophilic groups in biological environment and also exhibit antineoplastic activity.

Since Rosenberg and his coworkers^[1] reported the antitumor properties of *cis*-dichlorodiamineplatinum (*cis*-DDP), much work centered on the clinical use of *cis*-DDP leading to the licensing of it as an antitumor drug. The use of *cis*-DDP has been very complicated because of negative side effects including gastrointestinal, hematopoietic, immunosuppressive, auditory and renal disfunction^[2]. One way of overcoming some of these negative side effects is to limit the movement of *cis*-DDP through inclusion of it into a polymer thus prohibiting filtration of the polymer by kidneys, etc. Attachment of *cis*-DDP onto a preformed polymer was first done by Allcock and his coworkers. Their polymer derivatives exhibit inhibitory activity towards several tumors^[3].

The method used by Yoshinori Kidani^[4] for preparing dicarboxylate platinum complexes could be used to prepare the polymer derivatives of *cis*-platinum complexes. CPSMA and PAA were used as preformed polymers onto which *cis*-DDP and its analogues were attached. CPSMA or PAA was dissolved in alkaline solution and *cis*-DDP and its analogues were treated with silver nitrate in water so these *cis*-platinum complexes could be converted into water soluble species^[5]. The expected polymer derivatives could be obtained when the two aqueous solutions were brought together.

The reaction between the polymer derivatives of *cis*-platinum complexes and nucleophilic groups in biological environment was studied. Pt—O bond was easily attacked by nucleophilic groups. In other words, these polymer derivatives could release the pendent drugs in biological environment. The preliminary results showed that most of these polymer derivatives exhibit antineoplastic activity.

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EXPERIMENTAL

1. Materials and instrumentation

All reagents used for the synthesis of preformed polymers CPSMA and PAA were reagent grade and further purified according to the routine method. Styrene was washed first by 50% NaOH solution, then followed by running water, distilled water to remove the inhibitor and finally dried over anhydrous K_2CO_3 and distilled prior to use. Maleic anhydride was recrystallized from ether. Acrylic acid was redistilled before use.

IR spectra were obtained on Perkin Elmer 683. XPS were recorded on AEI ES-300. UV spectra were recorded on Jena Specord UV-VIS. Molecular weight were determined on Knauer Membrane Osmometer.

2. Synthesis of polymer carriers^[6,7]

Synthesis of CPSMA: 220 ml benzene and 24.5 g (0.25 mol) maleic anhydride were charged into a three-neck flask with a mechanical stirrer and a condenser. This was stirred slowly till all maleic anhydride was completely dissolved. To this was added 26.0 g (0.25 mol) styrene and 0.60g AIBN (azo bisisobutyl nitrile). This mixture was slowly heated till white precipitate appeared. At this time the temperature must be carefully controlled. After the completion of the reaction the solvent was removed by filtration. The crude product was washed by ether and dried in air. The product was dissolved in dry acetone and precipitated by benzene. The precipitate was filtered, washed by ether and dried in vacuum.

Elementary analysis of the product:

	C%	H%	O%
Calcd.	71.21	4.95	23.73
Found	70.23	4.84	25.02

$\bar{M}_n = 62,000$ (in acetone)

Synthesis of PAA: A 250 ml three-neck flask was equipped with mechanical stirrer and a condenser. To this was added 100 ml acetone, 15 g acrylic acid and 0.1 g AIBN. This was heated carefully under stirring. After the completion of the reaction, part of acetone was removed by rotavaporation and the concentrated PAA solution was cast into a tray and the solvent was evaporated by hot air. PAA film was cut into small pieces and thoroughly dried in vacuum.

Elementary analysis:

	C%	H%	O%
Calcd.	49.59	5.55	44.41
Found	48.62	5.03	46.01

$\bar{M}_n = 87,000$ (in acetone)

3. Preparation of cis-platinum complexes

Complexes of type *cis*-PtA₂X₂ (X=Cl, I; A=amine) were elegantly produced by an extension of Dhara's method for preparing *cis*-Pt(NH₃)₂Cl₂^[5]. One mole of K₂PtCl₄ was

dissolved in 30 ml water and 4 mole KI was added. This was heated on a steam bath till the solution became dark brown and then 1 mole diamine (or 2 mole amine) was added, heated again on a steam bath for 20 min for completion of the reaction. The dark yellow complexes were filtered, washed by ice water, cool ethanol and ether, dried in vacuum. Yield: >95%. The iodo-amine or diamine *cis*-platinum complexes are easily converted into chloro-complexes when they were first treated by AgNO_3 in water and then KCl.

4. *Synthesis of polymer derivatives of cis-platinum complexes*

400 mg (10 mmol) NaOH was dissolved in 30 ml water in a 250 ml flat-bottom flask with a spin-bar inside. To this was added ca. 1.01 g (5 mmol) CPSMA. This was heated on a magnetic stirrer with electric-hot plate till all CPSMA was completely dissolved.

5 mmol iodo or chloro-*cis*-platinum complexes was suspended in 15 ml water in a 100 ml beaker. To this was added 1.69 mg (10 mmol) AgNO_3 and stirred till the yellow color of the complex completely disappeared. AgCl or AgI was filtered. The pH value of the filtrate and CPSMA solution were carefully adjusted to 6.5–7.0 with NaOH and HNO_3 solution. Then they were mixed and the mixture was stirred for an hour and stood overnight. The pale yellow or white product was separated by centrifuge, washed with water, ethanol and ether. The products were dried in air, then in vacuum. Yield: 80–90%.

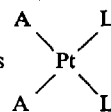
PAA derivatives of *cis*-platinum complexes were synthesized in the same way as CPSMA derivatives described above.

5. *Reaction between the polymer derivatives of cis-platinum complexes and nucleophilic groups in biological environment*

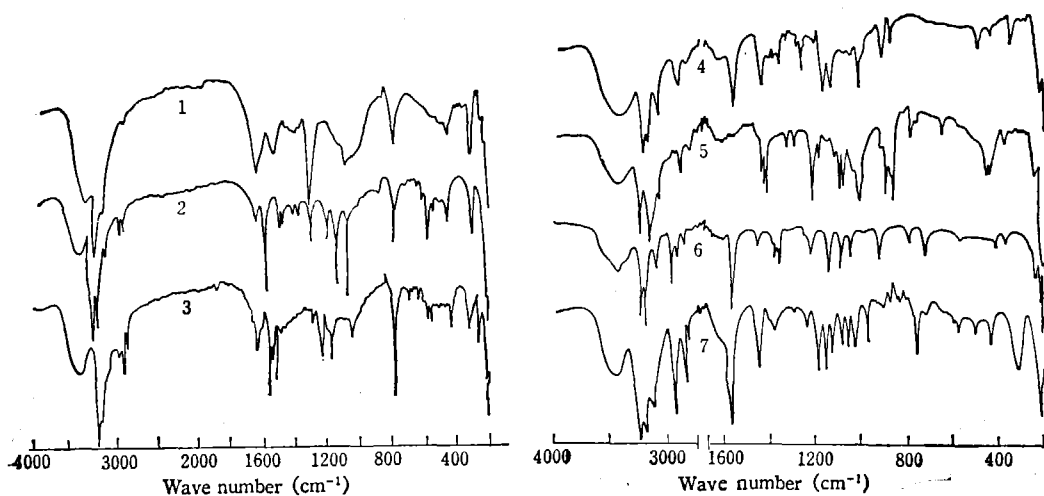
50 mg any polymer derivative was suspended in 8 ml $\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$ buffer (pH = 6.9) in a double-walled reactor with a spin-bar inside for stirring. To this was added glycine in excess. The reaction temperature was controlled at $37 \pm 0.5^\circ\text{C}$ by cycling water from a thermostat. After ca. 24 hrs, the reaction mixture was separated by centrifuge and the solution was subjected to UV-Visible spectra measurement.

RESULTS AND DISCUSSION

The *cis*-platinum complexes used in this experiment are given in Table 1 with their elementary analysis data. The IR spectra of these complexes are shown in Fig. 1. Generally speaking, these *cis*-platinum complexes with different amine ligands in Table 1 have better antitumor activity. The alternating copolymer CPSMA and PAA were prepared according to the literature^[6,7]. Their IR spectra are given in Fig. 2 and Fig. 3.

Table 1 Elementary analysis data for *cis*-platinum complexes

No.	Ligand A	Ligand L	C %		H %		N %		Cl %	
			calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	NH ₃	Cl			2.00	2.02	9.33	9.22	23.67	23.79
2		Cl	7.43	7.46	2.45	2.45	8.59	8.51	21.74	21.76
3		Cl	19.25	19.26	2.14	2.16	7.48	7.45	18.95	18.98
4		I	6.88	6.88	1.91	1.85	5.35	5.28		
5		I	8.97	9.00	1.87	1.84	5.23	5.06		
6		I	12.70	12.42	3.17	3.14	4.74	4.69		
7		I	12.80	13.20	2.49	2.54	4.97	4.88		

**cis*-1,2-diaminocyclohexane.Fig. 1 IR spectra of *cis*-platinum complexes in Table 1

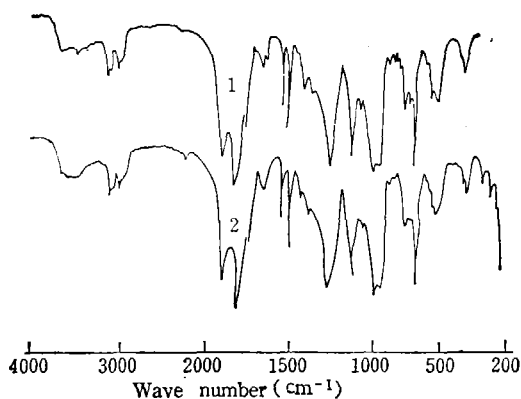


Fig. 2 IR spectra of poly(styrene-co-maleic anhydride):

1. Standard spectrum from "Atlas of Polymer and Plastic Analysis", 2nd Ed., Vol. 1, p. 304.
2. The spectrum of the alternating copolymer used in this experiment.

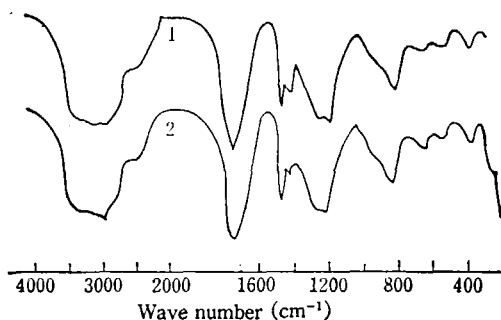


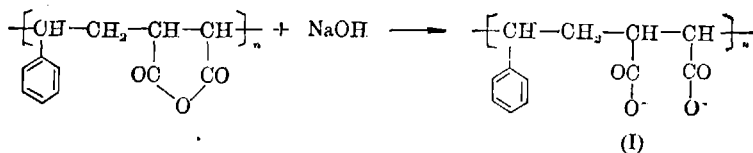
Fig. 3 IR spectra of polyacrylic acid:

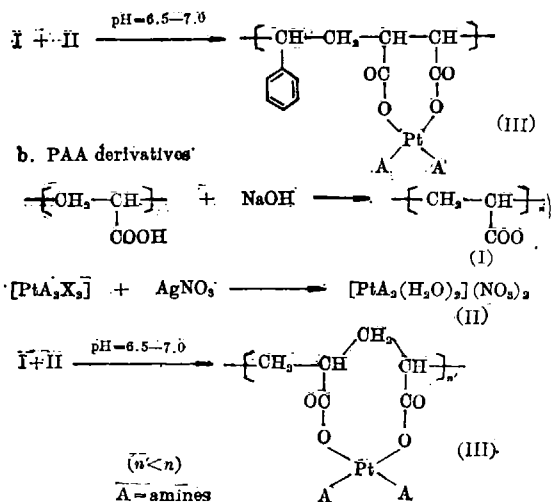
1. Standard spectrum from "Atlas of Polymer and Plastic Analysis", 2nd Ed., Vol. 1, p. 185.
2. The spectrum of polyacrylic acid used in this experiment.

All the samples in KBr.

The preparation of the polymer derivatives of *cis*-platinum complexes could be expressed in the following scheme:

a. CPSMA derivatives

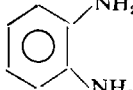
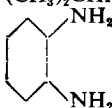




The acidity of the solution is very important for the reactions. When $\text{pH} > 8$ the more stable complex $\text{PtA}_2(\text{OH})_2$ may be formed so no reaction could be observed. When $\text{pH} < 6.0$ the polymer carriers were precipitated as acid, so the nitrogen content of the polymer derivatives (III) were very low and the IR spectra showed very strong $-\text{COOH}$ stretch at 1700 cm^{-1} .

It is interesting to know whether the $[\text{PtA}_2(\text{H}_2\text{O})_2]^{2+}$ ion could crosslink two chains of the preformed polymer or react with non-neighboring carboxylic groups of the same chain. From the observation of the reaction between $[\text{PtA}_2(\text{H}_2\text{O})_2]^{2+}$ and dicarboxylates only one sort of products could be separated, that is, dicarboxylates as a chelating ligands coordinated to Pt. So we believe that the main products should be (III) in the above scheme.

Table 2 N_f/N_c of the two series of polymer derivatives

No.	N_f/N_c %	No.	N_f/N_c %	Amines
PAA-1	83.19	P-1	80.00	NH_3
PAA-2	82.04	P-2	87.02	$\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$
		P-3	77.35	
PAA-3	75.40	P-4	74.16	
PAA-4	80.18	P-5	60.00	
PAA-5	82.43	P-6	87.27	$(\text{CH}_3)_2\text{CHNH}_2$
PAA-6	85.48	P-7	78.00	

N_f : N% found; N_c : N% calcd.

* *cis*-1,2-diaminocyclohexane.

Table 2 gives the ratio of actual and theoretical nitrogen content of the polymer derivatives. They are expressed in N_t/N_c . The IR spectra data of the polymer derivatives are listed in Tables 3 and 4.

It is not difficult to assign most of the bands of the IR spectra data of the polymer derivatives. Those very strong bands in $3420\text{--}3000\text{cm}^{-1}$ region are N—H stretching. The two rather weak bands at 550 and 465cm^{-1} are Pt—N stretching which shift with different amines. The —CO—O—CO— stretching of the copolymer at 1870 and 1750cm^{-1} in Fig. 2 disappeared in the spectra of the polymer derivatives in Table 3. A new one appeared at about 1600cm^{-1} which is —CO—O—M stretching (M=metal). The unionized or uncoordinated COO stretching band occurs at $1750\text{--}1700\text{cm}^{-1}$. Fig. 3 clearly shows COO stretching of PAA at 1700cm^{-1} and the —CO—O—M of PAA derivatives appears near 1600cm^{-1} showed in Table 4. Those weak bands at 1700cm^{-1} in Tables 3 and 4 are the unreacted COOH in the polymer carriers^[8].

Table 3 IR spectra data of CPSMA derivatives of *cis*-platinum complexes

Sample No.	Wave number (cm^{-1})				
P-1	3420s 1500w 1250w 760w 310w	3280vs 1450w 1090w 700s	2920s 1380s 1025w 600m	1700w 1350s 875w 530m	1600vs 1325s 800w 460m
P-2	3420vs 1710w 1390m 765m	3250vs 1630s 1290w 700s	3200s 1560s 1190w 565m	2920m 1500w 1125w 460m	2860w 1450w 1050m 310w
P-3	3420vs 1625w 1380w 760m	3200vs 1550w 1240m 700s	2915s 1500m 1175s 540m	2880m 1450m 1100w 460m	1700s 1400w 1030w 320w
P-4	3400vs 1620vs 1225w 870m 460w	3220vs 1550s 1160w 760m 320m	2920s 1480m 1125w 700s	2850m 1450s 1030w 590w	1700w 1380vs 930w 540w
P-5	3420vs 1620s 1220s 700vs	3200s 1550s 1090w 550m	3100s 1490s 1025w 460w	2910s 1380m 890s 320m	1710s 1320m 760m
P-6	3420vs 1720w 1380s 1070w 550m	3220s 1630s 1330w 930w 450m	2980s 1550s 1240w 760m 310w	2920s 1490w 1155w 700s	2840m 1450w 1110w 590w
P-7	3420s 1700w 1250w 1060w 760m 350w	3210s 1590vs 1200w 1030w 700vs 310w	3100s 1490m 1160w 980w 590m	2940vs 1450m 1130w 910w 550m	2860s 1380vs 1090w 880w 400w

vs=very strong; s=strong; m=medium; w=weak. (KBr tablets)

Table 4 IR spectra data of PAA derivatives of *cis*-platinum complexes

Sample No.	Wave number (cm ⁻¹)				
PAA-1	3420vs 1540vs 1165w 525m	3370vs 1448s 1100w 465m	2923s 1400s 1025m 260m	2842s 1385s 860w 250m	1640vs 1325s 780w
PAA-2	3430vs 1563vs 1053s 250m	3230vs 1450s 850w	2920s 1400vs 565m	2850m 1330m 480m	1640vs 1165m 260m
PAA-3	3463vs 1558vs 1220w 590w 260m	3234vs 1447vs 1035w 570w 229s	2945s 1386s 930w 465w 212vs	2840m 1300w 897w 290w 208s	1615vs 1250w 850w 265w
PAA-4	3432vs 1575s 1062w 239s	3180vs 1448m 667w	2924m 1395m 474w	2854w 1383s 288w	1623vs 1228m 259m
PAA-5	3450vs 1640s 1155s 815m 450m 230m	3200vs 1550s 1109s 775w 400w	2970s 1450s 1070m 660w 320w	2920vs 1385s 933s 620w 260m	2840s 1250m 850m 532w 245m
PAA-6	3452vs 1600vs 1250w 1060m 848w 440m 250m	3230s 1570s 1200w 1030m 770w 355m 230s	3098s 1550s 1165w 935w 660w 315w 215vs	2920vs 1448s 1142m 915w 588w 284w	2850s 1380vs 1090m 880w 515w 260w

vs=very strong; s=strong; m=medium; w=weak. (KBr tablets).

Although a lot of platinum complexes of carboxylic acids were reported in the literature, only a few authors studied the assignments of Pt—O vibrations. Fujita et al. assigned Pt—O stretching of $K_2Pt(OX)_2 \cdot 3H_2O$ (OX=oxalate) at 575, 559cm⁻¹. Kieft and Nakamoto^[9] assigned Pt—O stretching of bidentate glycine complex at 388cm⁻¹. In the region of 300—600 cm⁻¹ in Table 3 only one band at 320—310cm⁻¹ can be identified except two Pt—N bands. It is reasonable to consider the possible assignment, but we do not have enough evidence to assign the Pt—O absorption. It seems more difficult to assign the Pt—O absorption of PAA derivatives because the spectra are more complicated in the region below 600 cm⁻¹.

XPS data further confirmed the formation of bonding between platinum and the carboxy group. In comparison with CPSMA, the chemical shifts of O_{1s} and Pt_{4f_{7/2}} of its derivatives are ca. 0.6 eV and —0.6 eV respectively as shown in Fig. 4 and Fig. 5. Also, the chemical shifts of O_{1s} and Pt_{4f_{7/2}} of PAA derivatives are ca. 0.9 eV and —0.5 eV (Fig. 6 and Fig. 7) respectively.

The antitumor activity of *cis*-platinum complexes is considered to be due to the ligands exchange with biologically important molecules. The method we used for testing our polymer

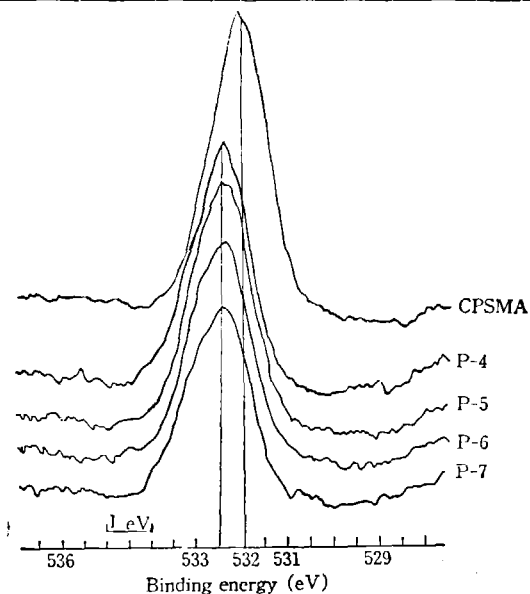


Fig. 4 O_{1s} XPS for CPSMA and CPSMA derivatives of *cis*-platinum complexes in Table 1

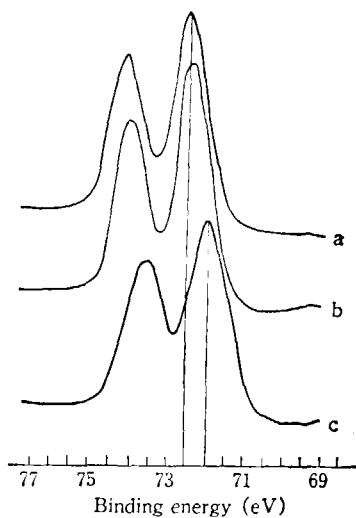


Fig. 5 Pt_{4f_{7/2}} XPS

- a. *cis*-dichloro(*cis*-diaminocyclohexane) platinum.
- b. *cis*-diiodo(*cis*-diaminocyclohexane) platinum.
- c. CPSMA derivatives of b.

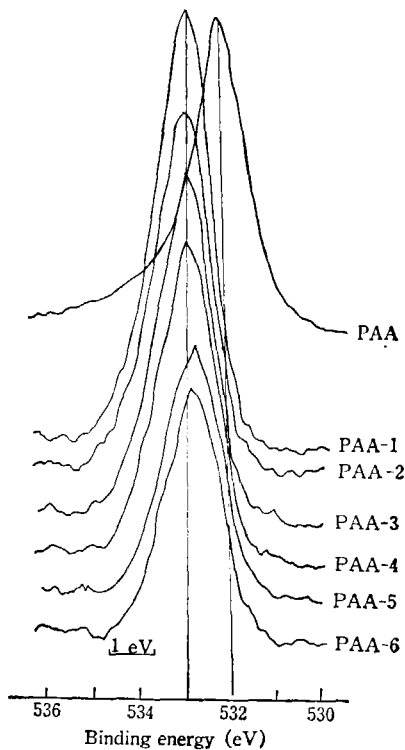


Fig. 6 O_{1s} XPS for PAA and PAA derivatives of *cis*-platinum complexes in Table 1

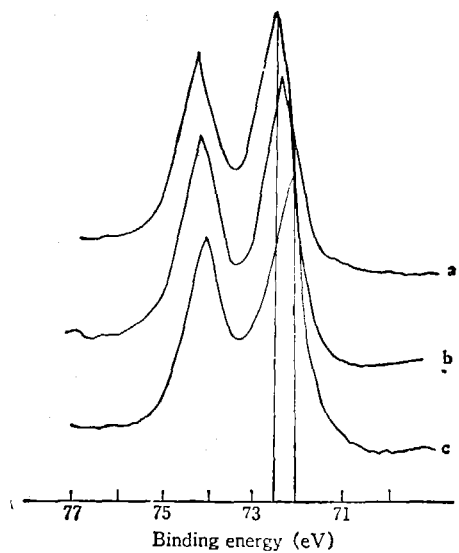


Fig. 7 Pt_{4f_{7/2}} XPS

- a. *cis*-dichloro(*cis*-diaminocyclohexane) platinum.
- b. *cis*-diiodo(*cis*-diaminocyclohexane) platinum.
- c. PAA derivatives of b.

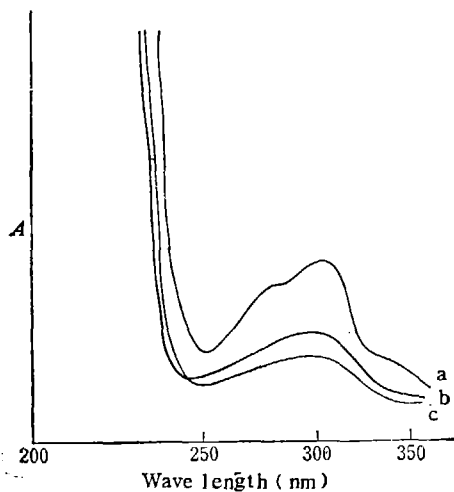


Fig. 8 a. UV spectrum of DDP.
b. UV spectrum of DDP plus glycine at 80°C for an hour.
c. UV spectrum of P-1 acted by excessive glycine at $37 \pm 0.5^{\circ}\text{C}$ and $\text{pH}=6.9$.
A=absorbance; random concentration; 1 cm path.

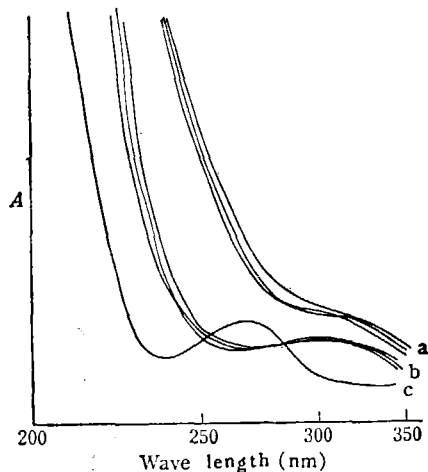


Fig. 9 a. UV spectra of different pH solutions of the polymer derivative E-7 acted by excessive glycine at $37 \pm 0.5^{\circ}\text{C}$, $\text{pH}=6.9$.
b. UV spectra of the reaction product of *cis*-dichloro(*cis*-1,2-diaminocyclohexane) platinum and different molar (1-3) glycine.
c. UV spectrum of *cis*-dichloro(*cis*-1,2-diaminocyclohexane) platinum.
A=absorbance; random concentration; 1 cm path.

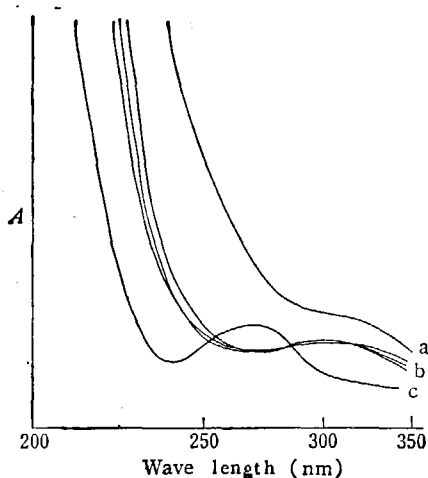


Fig. 10 a. UV spectrum of the polymer derivative PAA-6 acted by excessive glycine at $37 \pm 0.5^{\circ}\text{C}$, $\text{pH}=6.9$.
b. UV spectra of the reaction product of *cis*-dichloro(*cis*-1,2-diaminocyclohexane) platinum and different molar (1-3) glycine.
c. UV spectrum of *cis*-dichloro(*cis*-1,2-diaminocyclohexane) platinum.
A=absorbance; random concentration; 1 cm path.

derivatives is based on the one used by Pivcová et al.^[10]. From the results given in Fig. 8 to Fig. 10 we can reach the conclusion that the polymer derivatives are able to exchange ligands with nucleophilic groups in biological environment. The reaction products of polymer derivatives with glycine are colorless solutions. The absorption spectra do not depend on pH, so the new compounds should be $[\text{PtA}_2(\text{H}_2\text{NCH}_2\text{COOH})_2]^{3+}$.

Preliminary results showed that these polymer derivatives of *cis*-platinum complexes exhibit antineoplastic properties for several sorts of tumors. This will be reported later.

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