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Reaction of K_2PdCl_4 with Synthetic and Natural Nucleic Acids

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Abstract — Circular dichroism was used to study reaction of K_2PdCl_4 with polydeoxyribonucleotides and calf thymus DNA. The optical and structural characteristics of the molecular components were determined to show that K_2PdCl_4 reacts with natural and synthetic acids, producing profound rearrangements in their structure.

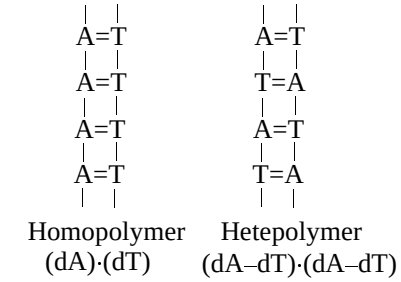
At present the significance of metal ions in the biochemistry of nucleic acids is quite obvious. Metal ions play a fundamental role in the structure and functions of nucleoproteids and exert effect on synthesis protein and transmission of hereditary information [1–4].

Most studies in this field have been focused on complex formation of nucleic acids with "biometals" [1–3], and, after the discovery of the antitumor activity of *cis*-diamminedichloroplatinum, with platinum compounds [5–10]. It is commonly accepted that platinum metals bind with nucleic acids in two steps. First a monodentate complex with a Pt–N^7 (guanosine, adenosine) and a Pt–N^3 (cytidine) bond is formed and then a chelate bidentate complex that disturbs DNA transcription and replication [9, 10].

Unlike platinum complexes, the information on reactions of palladium compounds with nucleic acids is scarce [11–15]. Shinshiashvili *et al.* [11] proposed, based on spectrophotometric characteristics and reduced characteristic viscosity of solutions, that Pd(II) ions induce single-chain scissions of the basic frame of native calf thymus DNA. According to the referees, the mechanism of DNA degradation involves hydrolysis of phosphodiester bonds in DNA via protonation of one or another of its groups [11]. Moreover, the ability of Pd(II) to form electrostatic bonds with phosphate groups and to react with guanine and adenine N^7 atoms, producing changes and distortions in the secondary structure of DNA, was discovered [12–14]. Pneumatikakis *et al.* [15], using ^1H NMR spectroscopy, studied the relative strengths of bonding of purine and pyrimidine nucleotides with Pd(II) via different heterocyclic atoms (N^1, N^3, N^7).

The recently discovered diverse biological, including cytotoxical, activity of Pd(II) compounds [16–18] has stimulated studies on the nature of their complexes with nucleic acids. In this connection the aim of the present work was to study reaction of K_2PdCl_4 with synthetic and natural polydesoxyribonucleotides.

We experimented with the following nucleic acids: poly[dA]·poly[dT], poly[dG]·poly[dC], poly[dA–dT]·poly[dA–dT], poly[dG–dC]·poly[dG–dC], and native calf thymus DNA. Here and hereinafter, the following abbreviations are accepted: (A) adenosine, (T) thymine, (G) guanosine, and (C) cytidine. The structural difference between homo- and heteropolymers is exemplified below by poly[dA]·poly[dT] and poly[dA–dT]·poly[dA–dT].



Homopolymer (dA)·(dT) Hetepolymer (dA–dT)·(dA–dT)

In homopolymers, each of the complemetary chains includes nucleotides of the same type, while hetepolymers include alternating complementary nucleotides in each of the chains.

The experimental method used was circular dichroism which has been widely employed for studying bioinorganic objects [3].

Reaction of K_2PdCl_4 with synthetic polydesoxyribonucleotides. With increasing K_2PdCl_4 : polynucleotide molar ratio, the circular dichroism spectra of the complexes of K_2PdCl_4 with poly[dA]·poly[dT] show decreasing amplitudes at 260 and 280 nm, a hypsochromic shift of the absorption maximum at

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Optical and structural characteristics of K_2PdCl_4 reaction with polydeoxyribonucleotides and calf thymus DNA

Characteristic[a]	Poly[dA]·poly[dT]	Poly[dG]·poly[dC]	Poly[dA–dT]·poly[dA–dT]	Poly[dG–dC]·poly[dG–dC]	Calf thymus DNA	A form of DNA	C form (DNA of T-2 even fag)
r_0	1.25	1.4	1.0	0.28	1.42	1.0	1.0
l_0	0.8	0.71	1.0	3.6	0.7	0.7–1.0	0.7–1.0
$\Delta\varepsilon$	4.32	4.3	4.0	4.3	4.3	4.0	4.0

[a] (r_0) Number of moles of K_2PdCl_4 bound with one mol of DNA base pairs; (l_0) number of base pairs per one K_2PdCl_4 molecule complexed with a polymer; and ($\Delta\varepsilon$) molar dichroic absorbance of a complex, l mol^{-1} cm^{-1}.

280 nm (by 5–10 nm), a strongly decreasing amplitude of the negative shoulder of the spectrum, and lack of isosbestic points (Fig. 1).

The reaction of K_2PdCl_4 with nucleic acids was estimated in terms of the following optical and structural characteristics: r_0, l_0, and $\Delta\varepsilon$ (l mol^{-1} cm^{-1}). The phenomenological criterion l_0 which denotes the number of base pairs per one K_2PdCl_4 molecule complexed with a polymer, is detemined from the titration curves and is the reciprocal of r_0 (r_0 is the number of moles of K_2PdCl_4 bound with one mol of DNA base pairs).

The stoichiometry (l_0) of the complex of Pd(II) with poly[dA]·poly[dT], determined from the dependence of the circular dichroism amplitude on the quantity of K_2PdCl_4, is 0.8 (see table). From this it follows that 5 molecules of K_2PdCl_4 bind with 4 base pairs.

The circular dichroism spectrum of the complex of Pd(II) with poly[dG]·poly[dC] (Fig. 2) shows a sharply decreased dichroism at 260 nm and two isosbestic points at 240 and 280 nm, implying that the coordination of Pd(II) with this homopolymer is more complicated in nature that with poly[dA]·poly[dT]. Actually, the titration curves have two inflexes at $r_0(1)$ 0.9 and $r_0(2)$ 1.4. Correspondingly, two $\Delta\varepsilon$ values are determined ($\Delta\varepsilon_1$ 1 and $\Delta\varepsilon_2$ 4.3 l mol^{-1} cm^{-1}), which, too, is evidence in favor of two types of coordination of Pd(II) with poly[dG]·

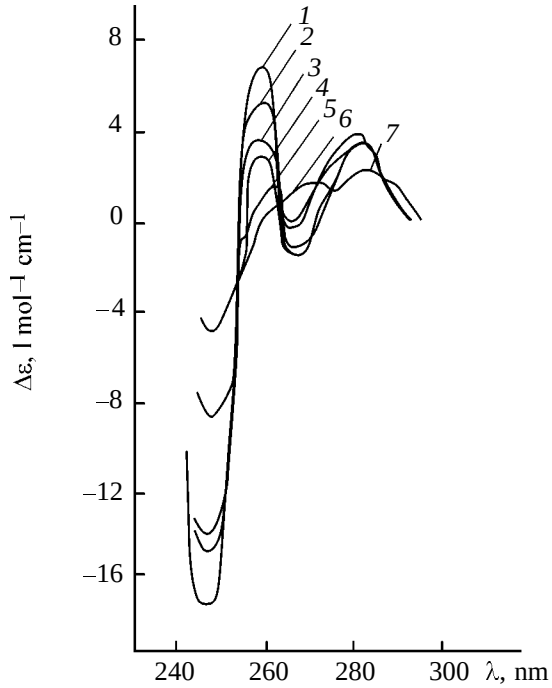


Fig. 1. Molar (reduced to 1 mol of nucleotide pairs) circular dichroism spectra of the complexes of K_2PdCl_4 with poly[dA]·poly[dT]. Molar concentration ratio of K_2PdCl_4 and DNA base pairs: (*1*) 0, (*2*) 0.34, (*3*) 0.69, (*4*) 1.04, (*5*) 1.37, (*6*) 1.73, and (*7*) 3.47.

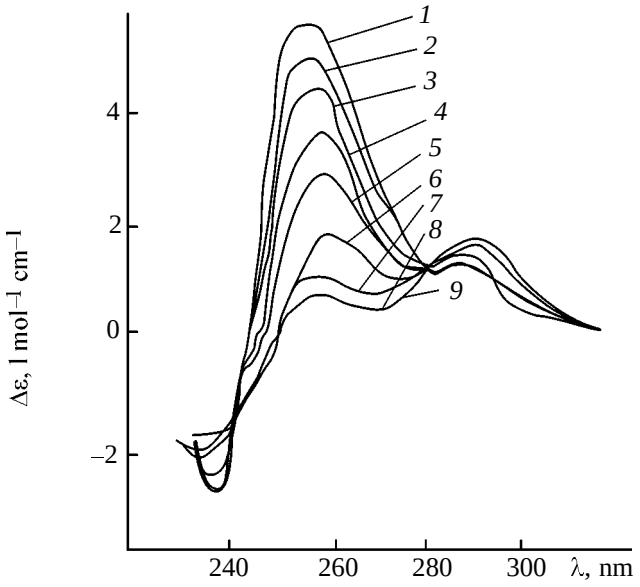


Fig. 2. Molar circular dichroism spectra of K_2PdCl_4 with poly[dG]·poly[dC]. Molar concentration ratio of K_2PdCl_4 and DNA base pairs: (*1*) 0, (*2*) 0.4, (*3*) 0.95, (*4*) 1.05, (*5*) 1.2, (*6*) 1.36, (*7*) 1.56, (*8*) 3.0, and 5.0 (*9*).

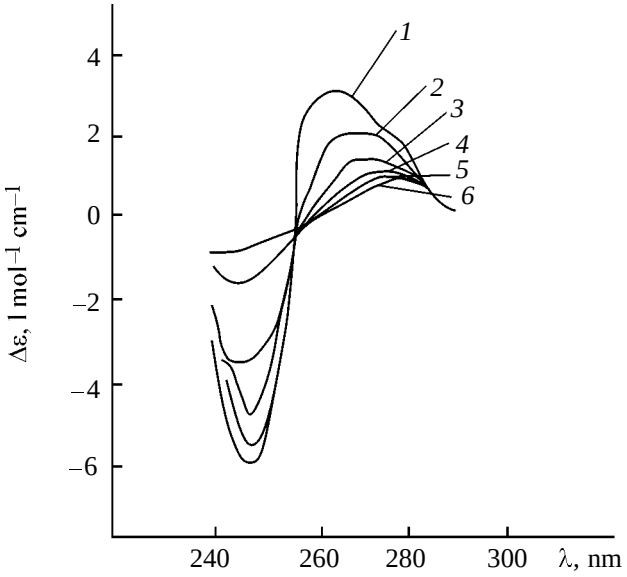


Fig. 3. Molar circular dichroism spectra of the complexes of K_2PdCl_4 with poly[dA–dT]·poly[dA–dT]. Molar concentration ratio of K_2PdCl_4 and DNA base pairs: (*1*) 0, (*2*) 0.31, (*3*) 0.62, (*4*) 1.25, (*5*) 2.5, and (*6*) 5.0.

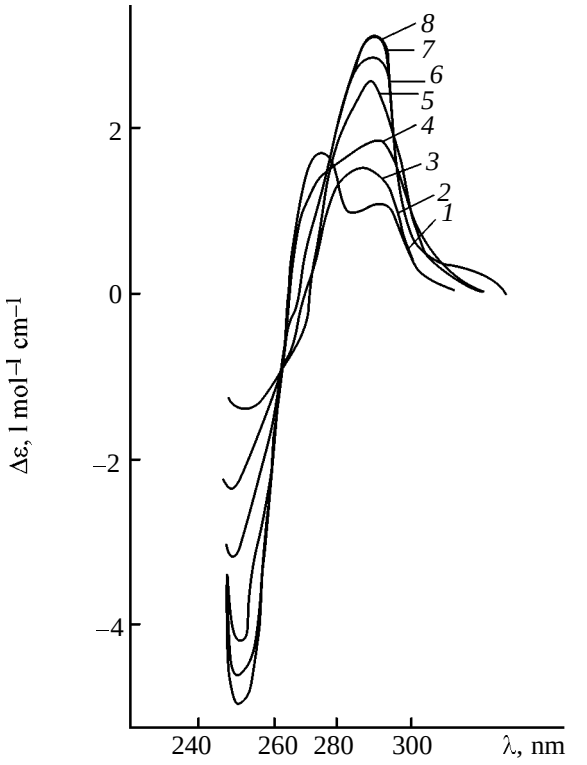


Fig. 4. Molar circular dichroism spectra of the complexes of K_2PdCl_4 with poly[dG–dC]·poly[dG–dC]. Molar concentration ratio of K_2PdCl_4 and DNA base pairs: (*1*) 0, (*2*) 0.06, (*3*) 0.12, (*4*) 0.22, (*5*) 0.36, (*6*) 0.46, (*7*) 0.6, and (*8*) 1.5.

poly[dC]. A similar coordination type ($\Delta\varepsilon$ 4.3 l mol^{-1} cm^{-1}) is characteristic of poly[dA]·poly[dT] (see table).

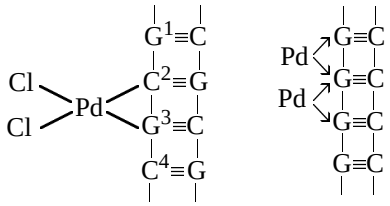
The stoichiometry of the complex of Pd(II) with poly[dG].poly[dC] corresponds to l_0 0.71, i.e. 3 molecules of K_2PdCl_4 bind with 2 base pairs of the polymer.

The circular dichroism spectra of the reaction mixtures of K_2PdCl_4 with poly[dA–dT]·poly[dA–dT] with various molar ratios show a single isosbestic point at 256 nm. Therewith, hypochromism and a bathochromic shift of the absorption maximum are observed (Fig. 3).

The stoichiometry of binding of Pd(II) with poly[dA–dT]·poly[dA–dT], determined from the circular dichroism amplitude, gives r_0 and l_0 values of 1 (see table), which corresponds to binding of 1 molecule of K_2PdCl_4 with 1 base pair of the polymer.

The circular dichroism spectra of the complex of Pd(II) with poly[dG–dC]·poly[dG–dC], obtained at various reactant ratios, form two isosbestic points at 262 and 276 nm. Therewith, a sharply enhanced hyperchromism of the positive amplitude at 285 nm and a decreased circular dichroism of the negative component are observed.

The stoichiometry of binding of K_2PdCl_4 with poly[dG–dC]·poly[dG–dC], determined from the intersection of asymptotes at the beginning and end of titration (Fig. 4), is l_0 3.6, i.e. 1 molecule of K_2PdCl_4 binds with 4 base pairs of the polymer.



The reaction of K_2PdCl_4 with poly[dG–dC]·poly[dG–dC] (the series of circular dichroism spectra form 2 isosbestic points) is apparently more intricate in nature that that with poly[dA–dT]·poly[dA–dT] (the series of circular dichroism spectra form 1 isosbestic point). Moreover, evidence for the different natures of binding of these polymers with K_2PdCl_4 comes from stoichiometric data (see table).

Thus, the reaction of K_2PdCl_4 with G- and C-containing homo- and heteropolymers is more intricate in nature than that with A- and T-containing polymers.

The l_0 value for all the polynucleotides, except for

poly[dG–dC]·poly[dG–dC], is ~1, which corresponds to binding of 1 molecule of K_2PdCl_4 with one DNA base pair. The molar dichroic absorbance is, regardless of the type of polynucleotide, 4.0–4.3 l mol^{-1} cm^{-1}, which points to a uniform mechanism of K_2PdCl_4 reaction with all the polynucleotides.

The results of recent studies on reaction of transition metals with nucleosides allow some suggestion as to the preferable binding sites of palladium ions with polydeoxyribonucleotides. In the case of purine bases, Pd(II) bind with the N^7 and N^1 atoms of adenosine, while in the case of pyrimidine nucleosides, with the N^3 atom [5, 9, 14, 19, 20]. Furthermore, strand cross linking in DNA, preventing its replication, can be proposed [5].

Reaction of K_2PdCl_4 with calf thymus DNA. When comparing the circular dichroism spectra of the complexes K_2PdCl_4 with calf thymus DNA at various reagent ratios, we noted the following phenomena: (1) hyperchromism at initial titration steps, that gives place to hypochromism at increasing concentration of K_2PdCl_4; (2) bathochromic shift of the thymus DNA dichroism maximum from 245 to 250 nm and from 275 to 290 nm; (3) isosbestic point at 261 nm; (4) decreased negative amplitude of the circular dichroism spectra (Fig. 5); and (5) l_0 0.7, i.e. 3 molecules of K_2PdCl_4 bind with 2 base pairs of DNA.

To gain a deeper insight into reaction of K_2PdCl_4 with DNA, we made use of nucleic acids with noncanonical structure, in particular, calf thymus DNA in the A form and the T2 even fag DNA with 30% of cytosine residues glucosylated by the wide groove (C form).

The A and B forms of DNA have different conformation of the sugar residues: the A form has a C_3-*endo* conformation of the sugar residues and the B form, C_2-*endo*. This difference gives rise to different geometric characteristics of the double helices, i.e. to different displacement of the pair with respect to the helix axis, different tilt angles, and different sizes of the minor and major grooves [21].

With increasing metal:nucleotide molar ratio, the circular dichroism spectra of the complexes of K_2PdCl_4 with noncanonical nucleic acids exhibit a hyproshromic effect and the bathochromic shift of the absorption maximum from 275 to 290 nm (Figs. 6a and 6b).

Thus, K_2PdCl_4 readily binds both with the classical B form and with the A and C forms of DNA, i.e. the complex formation is independent of the conformation of DNA.

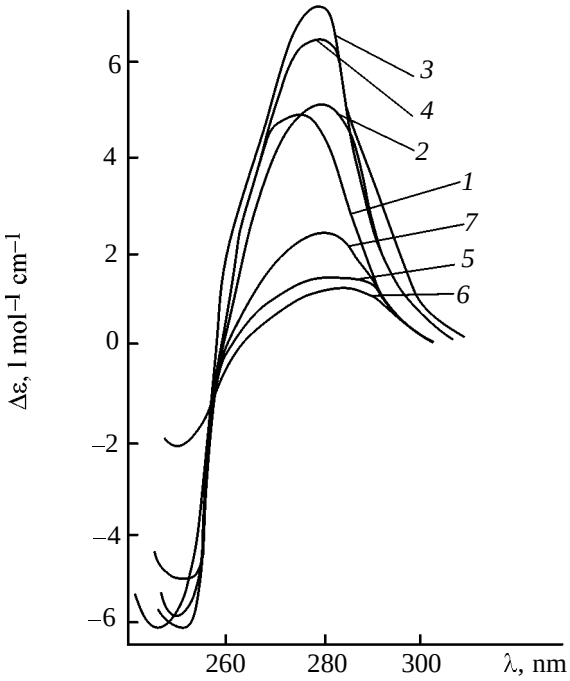


Fig. 5. Molar circular dichroism spectra of the complexes of K_2PdCl_4 with calf thymus DNA. Molar concentration ratio of K_2PdCl_4 and DNA base pairs: (*1*) 0, (*2*) 0.12, (*3*) 0.25, (*4*) 0.63, (*5*) 1.26, (*6*) 2.53, and (*7*) 5.05.

To conclude, Pd(II) coordinates to different homo- and heteropolynucleotides, as well as calf thymus DNA. However, circular dichroism does no allow determination of coordination centers. Nevertheless, relying on published data [14, 15, 22], we can propose that palladium complexes will most probably tend to attack more accessible sites, i.e. the N^1 or N^7 atoms of purine bases, that are closer to the polymer surface. The accessibility of these coordination sites, in its turn, is determined by the sizes of the grooves [14].

EXPERIMENTAL

The following samples of nucleic acids and synthetic polydeoxyribonucleotides were used: poly[dA]·poly[dT], E_{258}, poly[dG]·poly[dC], E_{258} 14 800, poly[dA–dT]·poly[dA–dT], E_{260} 13 600, and poly[dG–dC]·poly[dG–dC], E_{254} 16 800 l mol^{-1} cm^{-1}, [P.L. Biochemicals (USA)]; and T2 fag DNA (65% AT), E_{260} 13 200, and calf thymus DNA (58% AT), E_{260} 13 300 l mol^{-1} cm^{-1} (Sigma).

The synthetic polymers and DNA were dissolved in a buffer solution containing 10^{-4} mol l^{-1} of tris–HCl and 2×10^{-4} mol l^{-1} of NaCl, and subjected to

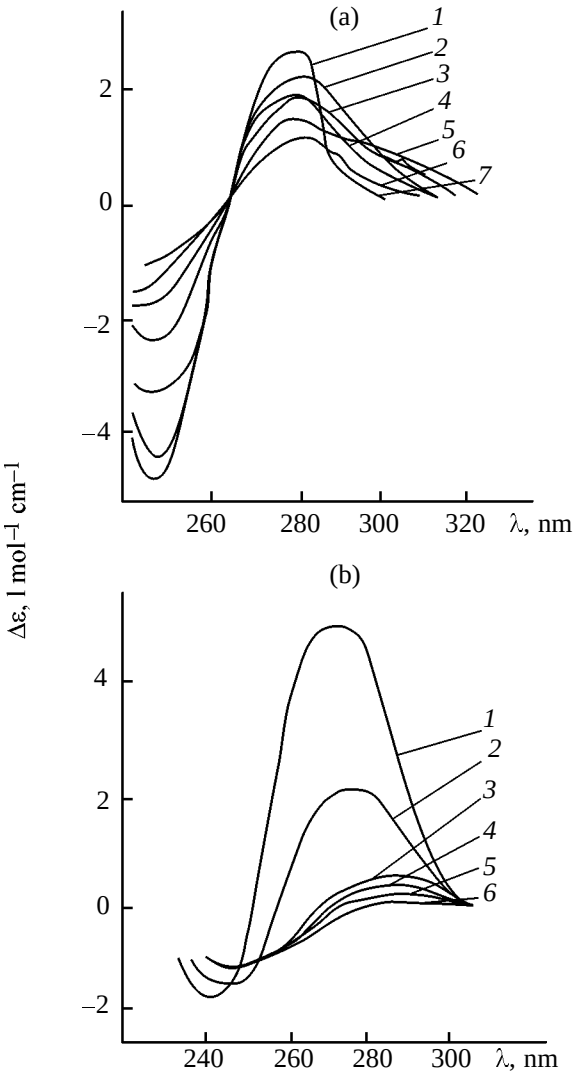


Fig. 6. Molar circular dichroism spectra (a) of the complexes of K_2PdCl_4 with T2 even fag DNA at molar concentrations ratios of K_2PdCl_4 and even fag base pairs of (*1*) 0, (*2*) 0.22, (*3*) 0.65, (*4*) 0.87, (*5*) 1.09, (*6*) 2.14, and (*7*) 4.32, and (b) of the complexes of K_2PdCl_4 with the A form of calf thymus DNA at molar concentration ratios of K_2PdCl_4 and DNA base pairs of (*1*) 0, (*2*) 0.2, (*3*) 0.35, (*4*) 0.61, (*5*) 1.0, and (*6*) 2.0.

dialysis against required buffer. The ionic strengths of the solutions were 10^{-4}–0.15 M NaCl.

The molar absorbance index was calculated per 1 mol of DNA base pairs. The concentrations of polynucleotides and complexes were determined on Beckman and Cary spectrophotometers (USA).

The circular dichroism spectra were taken on a Yobin Ivon Mark-III dichrograph (France).

K_2PdCl_4 exhibits optical activity only on binding with DNA. Therefore, having determined the optical activity ΔA of the compound of K_2PdCl_4 with DNA by circular dichroism, one can estimate the concentration of bound K_2PdCl_4 molecules per one pair of DNA bases (r) by the following formula:

$$r = \Delta A/(\Delta\varepsilon\, 0.5P).$$

Here ΔA is the circular dichroism value, $\Delta A = AS/l$, where A is the circular dichroism amplitude on the recorder of the instrument, mm; S is the sensitivity of the dichrograph, mm^{-1}; l is the length of the cell, cm; $0.5P$ is the molar concentration of DNA in the solution, M; $\Delta\varepsilon$ is the molar dichroic absorbance of the complex, $l\ mol^{-1}\ cm^{-1}$, $\Delta\varepsilon = \Delta A/c_{calc}$, where c_{calc} is an index calculated from the initial part of the titration curve, when the added complex compound binds with the nucleic acid, $c_{calc} = (c_{init}\Delta V)/(V_0 + \Delta V)$ (where c_{init} is the initial concentration of the complex compound, M, ΔV is the added volume of the complex compound, μl, and V_0 is the initial volume of the cell, μl).

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