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Study of the ionic equilibriums in aqueous solutions and coordination properties of *Adefovir* and *Cidofovir* used as antiviral drugs

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ABSTRACT

The dissociation constants of *Adefovir* ({[2-(6-amino-9H-purine-9-l)ethoxi]methyl}phosphoric acid) used in the treatment of hepatitis B and *Cidofovir* ({[2-(6-amino-9H-purine-9-l)ethoxi]methyl} phosphoric acid), used in the treatment of *cytomegalovirus* retinitis in AIDS patients, together with the stability constants of its Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}, and Mg^{2+} complexes were studied by potentiometric titration. *Adefovir* and *Cidofovir* were abbreviated to PMEA and HPMPC. All measurements were obtained under two sets of conditions: at 298 K and ionic strength (I) of 0.1 mol dm^{-3} (NaCl) and at 310 K and I of 0.16 mol dm^{-3} (NaCl), corresponding to the conditions of human blood. Equilibrium constants in aqueous solution were calculated using the program SUPERQUAD. Also, solvent effects on the equilibrium constants of the ligands were examined. Moreover, all coordination studies were carried out with the ligand/metal molar ratios of 1:1. This ligand/metal stoichiometric ratio was spectrometrically determined using Job's method.

1. Introduction

Viruses cause many diseases such as AIDS, hepatitis, Ebola, herpes, influenza and rabies. Treatment of such diseases is difficult; because, antibiotics do not affect viruses. Today, the some viral diseases are treated immune system stimulation with interferon or nucleotide analogs in the form of suppression of viral replication [1]. The basic chemical structure of the acyclic nucleotide analog (ANP) compounds consists of a purine base (i.e., adenine, guanine, or 2,6-diaminopurine) or pyrimidine base, attached to an acyclic side chain that ends in a phosphonate group. The strong C—P bond is chemically and enzymatically stable, thus preventing hydrolysis of the ANP compounds in biological systems. Based on the antiviral activity spectrum, the ANP compounds can be divided in the following subclasses [2]. The highly anionic charge of the phosphonate moiety of the ANP compounds makes their cellular uptake rather inefficient.

Adefovir [{[2-(6-amino-9H-purine-9-yl)ethoxi]methyl} phosphoric acid] (PMEA) and *Cidofovir* (S)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (HPMPC) (see Fig. 1) are nucleoside phosphonate analogs, a class of novel antivirals structurally related to natural nucleotides [3,4] and they were shown to enter the cells by endocytosis, a process that is marked by slow kinetics and temperature dependence [5,6]. Adefovir dipivoxil, an orally available prodrug of PMEA, is currently undergoing clinical evaluation as an anti-HIV and anti-hepatitis B virus agent [7–11]. HPMPC is an antiviral nucleotide analog [12–14] with potent in vitro and in vivo activity against human cytomegalovirus (HCMV) retinitis in AIDS patients [15–17] and other herpesviruses [18–21].

As a result of the above, numerous studies have been done on the antiviral activities of PMEA and HPMPC. However, theirs ionic behaviors in aqueous solution and coordination properties have not been explained sufficiently, whereas many articles have been published related to the electro-analytical properties of similar compounds using potentiometric methods [22–26]. Proton transfer reactions of PMEA and HPMPC are very important for explaining its coordination properties and biological activity. Thus, in this work, the dissociation constants of the ligands, and the stability constants of its complexes with Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}, and Mg^{2+} ions were studied potentiometrically. This is a very useful electro-analytical method [27].

2. Materials and methods

2.1. Reagents

$CuCl_2$, $NiCl_2$, $ZnCl_2$, $CoCl_2$, $CaCl_2$, $MgCl_2$, and NaCl used in this research were purchased from Merck. All reagents were of analytical quality (≥98%) and were used without further purification. Potassium hydrogen phthalate (KHP) and borax ($Na_2B_4O_7$) were purchased from Fluka. For calibration of the electrode,

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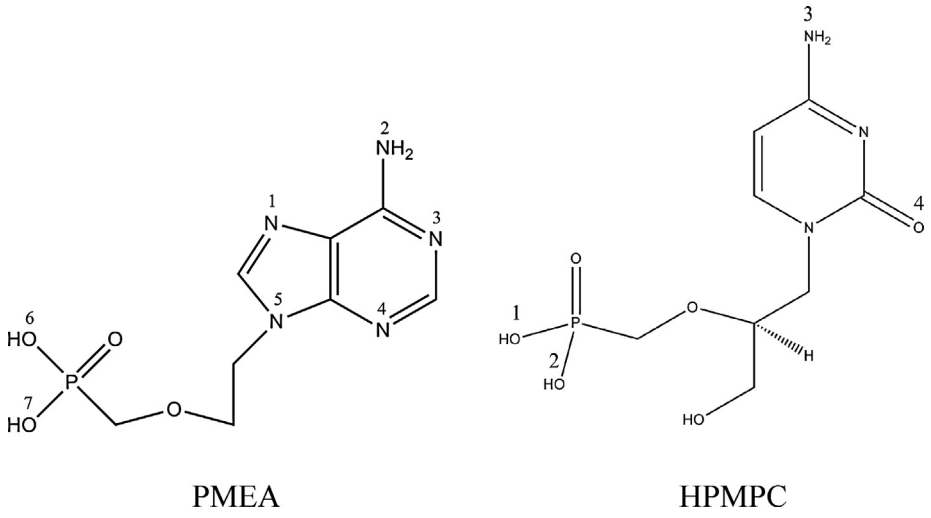

Fig. 1. Chemical structures of the ligands.

0.05 mol kg^{-1} KHP and 0.01 mol kg^{-1} borax were prepared. PMEA (purity 99%) is purchased from Watson International Ltd. HPMPC (purity 98%), ethyl alcohol, 0.1 mol dm^{-3} NaOH, and 0.1 mol dm^{-3} HCl as standard were purchased from Aldrich. Solutions of metals ions (1×10^{-3} mol dm^{-3}) were prepared from $CuCl_2$, $NiCl_2$, $ZnCl_2$, $CoCl_2$, $CaCl_2$, and $MgCl_2$ and standardized with ethylenediaminetetraacetic acid (EDTA) [28]. A PerkinElmer Lambda 35 UV/vis spectrometer was used for determination of the HPMPC-metal stoichiometric ratio. CO_2-free double-distilled deionized water was used throughout the experiments, which had been obtained using an aquaMAX™-Ultra water purification system (Young Lin Inst.) whose resistivity was 18.2 MΩ cm^{-1}. All experiments were conducted with a Molspin pH meter™ that was connected to an automatic buret with an Orion 8102BNUWP ROSS Ultra combination pH electrode. Temperature of the titration cell was controlled by a thermostat (DIGITHERM 100, SELECTA) and the cell solution was stirred constantly during the experiments.

2.2. Procedures

A 1×10^{-3} mol dm^{-3} solution of the ligands in water was prepared and 0.01 mmol of each was placed in the cell. Then 0.1 mol dm^{-3} NaOH and 0.1 mol dm^{-3} HCl as standard were used. An ionic background of 1.0 mol dm^{-3} NaCl stock solution was prepared to maintain constant ionic strength. Then 0.03 mmol acid solution was added from 0.1 mol dm^{-3} HCl for withdrawing to an initial pH of approximately 3. Nitrogen gas (99.9%) was purged through the cell solutions constantly to exclude atmospheric CO_2 during all experiments. Solutions of complex titration were prepared with the same quantities of components. Additionally, 0.01 mmol of each metal ion, including $CuCl_2$, $NiCl_2$, $ZnCl_2$, $CoCl_2$, $CaCl_2$, and $MgCl_2$ solutions, was added. All experiments were carried out at the molar ratio of 1:1 ligand to metal and they were carried out at both 298 K, *I*: 0.1 ionic strength and 310 K, *I*: 0.16 ionic strength. The electrode was calibrated according to the instructions in the Molspin manual [29], with buffer solutions of pH 4.005 (KHP) and pH 9.180 (borax) at 298 K, and pH 4.022 (KHP) and 9.088 (borax) at 310 K [30] in aqueous solution. The summary of the titration conditions are given in Table 1. The program SUPERQUAD [31] was used for the determination of equilibrium constants. The pH data (115–135) were obtained after the addition of 0.03 cm^3 increments of 0.025 mol dm^{-3} NaOH solutions.

But, in Section 3.2, mixed solution was used in the experiments. Therefore, the potentiometric cell was calibrated before each experiment to obtain the pH values for the solvent mixture studied [32,33]. For this purpose, the HCl solutions prepared in each medium with titrated NaOH solutions. For all the solvent mixtures examined, reproducible values of the autoprotolysis constants K_w were calculated from several series of $[H^+]$ and $[OH^-]$ measurements in 0.10 mol dm^{-3} NaCl [34,35]. During each titration the ionic strength was maintained at 0.1 mol dm^{-3} NaCl and a potential reading were taken after a suitable time (normally 2–3 min) for each equilibration.

3. Results and discussion

3.1. Dissociation constants

All measurements for the calculation of dissociation constants of the ligands were obtained potentiometrically in about room (*I*: 0.1 mol dm^{-3} of NaCl at 298 K) and human blood (*I*: 0.16 mol dm^{-3} of NaCl at 310 K) conditions. If LH_7^{5+} and LH_4^{2+} denotes the fully protonated forms of PMEA and HPMPC (see Fig. 10a), theirs deprotonation equilibriums are as follows:

$$LH_n + H_2O \rightleftharpoons LH_{n-1} + H_3O^+ \quad (1)$$

In each stage, one proton dissociates and dissociation constants (K_n; n = 1–7) are given as;

$$K_n = \frac{[LH_{n-1}] \cdot [H_3O^+]}{[LH_n]} \quad (2)$$

Hence, the titration curves for the ligands in water with NaOH as a titrant are shown in Fig. 2.

HPMPC has two parts. One of these parts is cytosine, which is a nucleic base and the most basic atom is 4O within cytosine. It is assumed that high alkalinity causes the protonation of the oxygen atom in the 4 position on the ligand and increases the mobility of its π-electrons. In solutions more alkaline than pH 9, the oxygen atom in the 4 position begins to be hydrolyzed at appreciable ratios [36,37]. Therefore, electron pairs that are on the sp^3 nitrogen of cytosine participate in resonance and this is caused by the ring of cytosine having an aromatic character. Consequently, ketone form was easily transformed into enol form depending on pH (see Fig. 3). Therefore, cytosine has two ionizable groups: enol in the 4 position and aniline in the 3 position. Other part of PMEA is adenine that is nucleic base. It has got five protonable nitrogen atoms. Therefore, six pK_a values are obtained from this ligand.