Potentiometric Study of Interaction of Glutarimide, *N*-Phenylacetyl-L-glutamine, and *N*-Acetyl-L-glutamine with a Series of Metal Ions

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The interaction of glutarimide, *N*-phenylacetyl-L-glutamine, and *N*-acetyl-L-glutamine with Cu(II), Ni(II), Co(II), Zn(II), Mn(II), Cd(II), Pb(II), Mg(II), and Ca(II) ions has been investigated by a potentiometric method. It is concluded that *N*-phenylacetyl-L-glutamine and *N*-acetyl-L-glutamine are unable to form stable complexes with the above metal ions in solution on varying the pH values, which is important in view of the pharmacological application of these compounds.

Glutarimide forms complexes with Cu(II), Zn(II), Co(II), and Ni(II) ions. The stability constants of the 1 : 1 and 1 : 2 metal—glutarimide complexes have been determined at 45 °C and 0.10 mol dm^{-3} (KNO_3) ionic strength.

Glutarimide is the biologically active centre present in several new anticancer agents introduced recently into the experimental clinical chemotherapy: Antineoplaston A10 [1, 2], PCNU [3], and Aminoglutethimide [4]. The glutarimide antibiotics inhibit protein synthesis and exert antitumour and fungicidal activities [5—7].

N-Phenylacetyl-L-glutamine, a new experimental drug, is the derivative of Antineoplaston A10 and it shows interesting anticancer activity and no toxicity [8]. *N*-Acetyl-L-glutamine has been reported to improve memory efficiency [9].

Since some of these drugs, *e.g.* Antineoplaston A10 and *N*-phenylacetyl-L-glutamine are administrated to patients in quite large doses [10], the problem arises whether these drugs are able to bind with the bivalent metal ions under the physiological conditions and thereby deplete important metal ions from the organism.

The aim of this study was to examine the interaction of glutarimide, *N*-phenylacetyl-L-glutamine, and *N*-acetyl-L-glutamine with the following metal ions: Cu(II), Ni(II), Co(II), Zn(II), Mn(II), Cd(II), Pb(II), Mg(II), and Ca(II). The studies were performed by potentiometric method at 25 °C and 45 °C under the constant ionic strength 0.10 mol dm^{-3} (KNO_3).

EXPERIMENTAL

Glutarimide was purchased from the Aldrich Chemical Co. and *N*-acetyl-L-glutamine was received from the Sigma Chemical Co. *N*-Phenylacetyl-L-glutamine was a generous gift from the Burzynski Research Institute, Texas, Houston.

Metal salts were of anal. grade purity. The initial 0.1 M solutions of Cu(II), Ni(II), Co(II), Zn(II), Mn(II), Cd(II), Pb(II), Mg(II), and Ca(II) were prepared by dissolving the metal salts in water (in the case of cadmium nitrate and manganese chloride a proper amount of HNO_3 and HCl, respectively, was added to avoid a hydrolysis of the metal ion).

The concentration of the metal ions was determined by the complexometric titration with EDTA in the presence of the appropriate indicator [11, 12]. Murexide was used for determination of copper, nickel, cobalt, and calcium. Eriochromate steam black was applied for zinc, magnesium, cadmium, and lead, whereas pyrocatechol violet was used for determination of manganese.

The aqueous solution of each ligand (0.10 M) was freshly prepared before each titration. Carbonate-free sodium hydroxide (0.10 M) was prepared and standardized by titration with potassium hydrogen phthalate.

The experimental method consisted of the potentiometric titration of each ligand with standard sodium hydroxide solution in the presence and absence of the metal ions being investigated.

An OP-211 model pH-meter (Radelkis, Budapest) with combination glass electrode was used to determine hydrogen ion concentration. The electrode system was calibrated with 0.05 M potassium hydrogen phthalate solution (pH = 4.00 ± 0.01) and a buffer (pH = 9.24 ± 0.01) from Electronic Instruments. The accuracy of the measured pH was ± 0.01. Measurements were also performed in the aqueous methanol solution (φ = 50 vol. %). In this case an additional calibration of the electrode system was made. According to the method described by *Van Uitert* and

Table 1. Acid Dissociation Constants of *N*-Acetyl-L-glutamine and *N*-Phenylacetyl-L-glutamine in Aqueous Solution and in the Water—Methanol Mixture (φ_r = 55/45), $I(KNO_3)$ = 0.10 mol dm^{-3}, θ = (25 ± 0.1) °C

Solvent	*N*-Acetyl-L-glutamine		*N*-Phenylacetyl-L-glutamine	
	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}
H_2O	3.52	12.18	3.70	11.94
H_2O—CH_3OH	4.36	12.43	4.52	12.66

Table 2. Acid Dissociation Constants of Glutarimide and Stability Constants of Glutarimide—Metal Complexes in Water and in the Water—Methanol Mixture (φ = 50 vol. %), $I(KNO_3)$ = 0.10 mol dm^{-3}, θ = (45.0 ± 0.1) °C

Solvent	pK_a	Metal ion	log K^{M}_{ML}	log $K^{ML}_{ML_2}$	|Δ log K|
100 % H_2O	10.54	Cu(II)	7.40 ± 0.01	6.56 ± 0.01	0.84 ± 0.02
		Zn(II)	5.79 ± 0.01	5.35 ± 0.01	0.44 ± 0.02
		Co(II)	4.87 ± 0.06	4.27 ± 0.06	0.60 ± 0.12
		Ni(II)	4.37 ± 0.01		
φ = 50 vol. % H_2O—CH_3OH	11.54	Cu(II)	7.96 ± 0.05	7.52 ± 0.05	0.44 ± 0.10
		Zn(II)	7.07 ± 0.03	6.68 ± 0.03	0.39 ± 0.06
		Co(II)	5.95 ± 0.02	5.55 ± 0.02	0.40 ± 0.04
		Ni(II)	5.77 ± 0.02		

Haas [13] the absolute calibration coefficient, μ°_{H}, for the water—methanol mixture has been determined. The values of μ°_{H} show a good agreement with those reported by *Lahiri* and *Aditya* [14] and *Bates* [15]. In calibration the HCl solutions of the concentration varying from 1.0 × 10^{-5} to 1.0 × 10^{-2} mol dm^{-3} have been used. A linear relationship has been found between the measured pH values and the actual concentration of the hydrogen ions in the pH range 2.5—12 in the mixed solvent. This dependence is parallel to that obtained from titrations in water without methanol. Therefore this electrode system could be employed for the mixed solvent in the pH range investigated.

Titrations of the investigated systems were performed at (25.0 ± 0.1) °C and (45.0 ± 0.1) °C and the ionic strength (KNO_3) was maintained constant at 0.10 mol dm^{-3}. In the titrated samples the concentration of *N*-acetyl-L-glutamine and *N*-phenylacetyl-L-glutamine was equal: 2.0 × 10^{-3} mol dm^{-3} as well as 1.0 × 10^{-2} mol dm^{-3}, whereas the concentration of the metal ions was varied as follows: 2.0 × 10^{-3} mol dm^{-3}, 2.5 × 10^{-3} mol dm^{-3}, 4.0 × 10^{-3} mol dm^{-3}, and 1.6 × 10^{-2} mol dm^{-3}. The concentration of glutarimide in the titrated samples was 1.0 × 10^{-2} mol dm^{-3} and that of the metal ions was varied: 0.5 × 10^{-2} mol dm^{-3}, 1.0 × 10^{-2} mol dm^{-3}, and 2.0 × 10^{-2} mol dm^{-3}. All metal to ligand mole ratios were tested by at least three titrations.

The nonlinear regression method [16] has been used to obtain the formation curves and the stability constants of the metal—glutarimide complexes. Only those data were used in calculations which has been measured in homogeneous solution with no precipitation. Computations were performed on PC 386.

RESULTS AND DISCUSSION

The acid dissociation constants, K_{ai}, for *N*-phenylacetyl-L-glutamine, *N*-acetyl-L-glutamine, and glutarimide were calculated from the following equation

$$pK_{ai} = pH + \log \frac{(1 - a + n - i)c_{HnL} - [H^+] + [OH^-]}{(a - n + i)c_{HnL} + [H^+] - [OH^-]}$$

where a is the titration fraction (the number of moles of base added per mole of ligand), n is the basicity of the ligand, c_{HnL} is the total concentration of the protonated form of ligand [17, 18].

The values of the calculated dissociation constants for *N*-acetyl-L-glutamine, *N*-phenylacetyl-L-glutamine, and glutarimide are shown in Tables 1 and 2.

To determine the stability constants of the metal complexes with the above ligands titrations were performed for the protonated form of the ligand and for the mixtures containing the protonated ligand and the metal ion at different metal : ligand mole ratios.

In the pH range 5—9 the titration curves for the protonated forms of *N*-acetyl-L-glutamine and *N*-phenylacetyl-L-glutamine can be almost exactly superimposed on the corresponding titration curves of these ligands in the presence of the following metal ions: Cu(II), Ni(II), Co(II), Zn(II), Mn(II), Cd(II), Pb(II), Mg(II), and Ca(II). The same result was obtained for all metal to ligand mole ratios used, including those cases in which relatively large excess x_r = (8 : 1) of metal ions was applied.

The measurements were performed in water and in the water—methanol mixture (φ = 50 vol. %) and

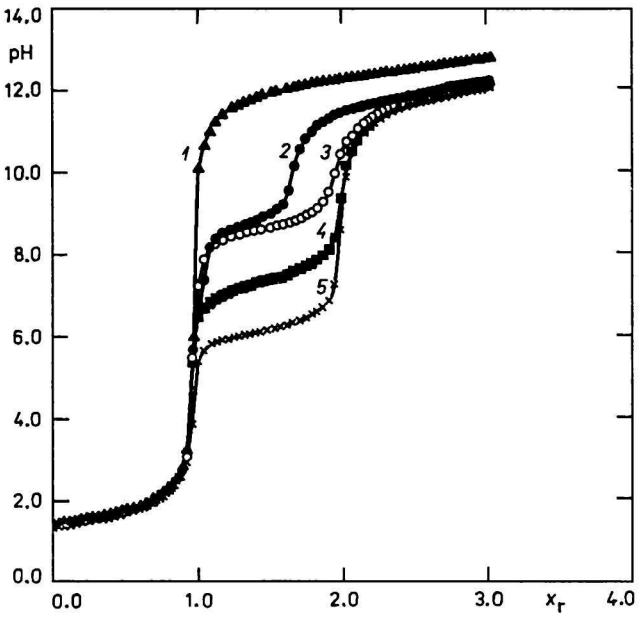

Fig. 1. Potentiometric titration curves of glutarimide and glutarimide—metal ion in water. The concentrations of the ligand and metal ions are: 1.0×10^{-2} mol dm^{-3} and 0.5×10^{-2} mol dm^{-3}, respectively. *1.* Glutarimide (G) in the absence of metal ion; *2.* G with Ni(II); *3.* G with Co(II); *4.* G with Zn(II); *5.* G with Cu(II). x_r = *n*(base)/*n*(metal ion).

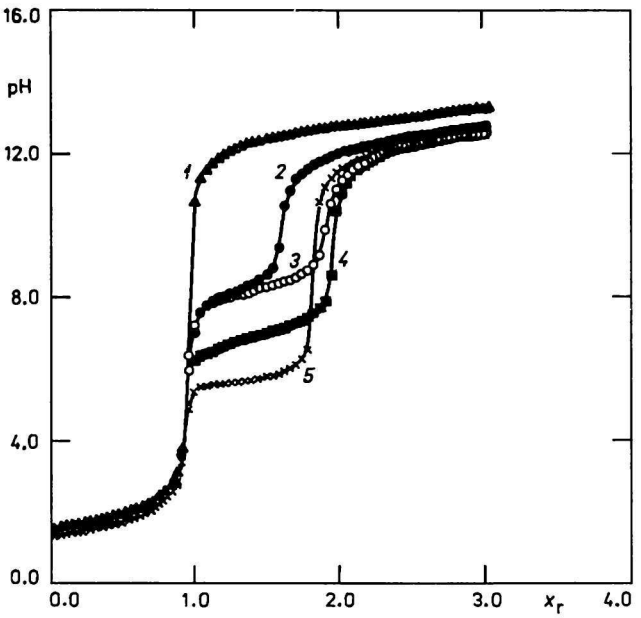

Fig. 2. Potentiometric titration curves of glutarimide and glutarimide—metal ion in aqueous methanol (φ = 50 vol. %). *1.* Glutarimide (G) in the absence of metal ion; *2.* G with Ni(II); *3.* G with Co(II); *4.* G with Zn(II); *5.* G with Cu(II). The metal to ligand mole ratio is 1 : 2. x_r = *n*(base)/*n*(metal ion).

the results obtained in both solvents were very similar. The aqueous methanol was used because of the suggestion [19] that the Cu(II) complex with *N*-acetyl-L-glutamine might be more stable in this mixture. It follows from our data, however, that no metal complexes are formed between the investigated bivalent metal ions and *N*-phenylacetyl-L-glutamine or *N*-acetyl-L-glutamine in these solvents, which is an important result in view of the pharmacological application of these compounds.

A shift of the titration curves was observed in the case of glutarimide in the presence of the Cu(II), Zn(II), Co(II), and Ni(II) ions. The shift of these curves (with respect to the titration curve of the free ligand) is seen in the pH range 5—9, in both solvents, as demonstrated in Figs. 1 and 2. To facilitate comparison of the stability constants of metal complexes with glutarimide, uridine [20], uracil and thymine [21], the measurements in this work were performed under the same conditions of temperature (45 °C) and ionic strength (0.1 mol dm^{-3} (KNO_3)) as those used in the other studies [20, 21]. The concentration of the bound ligand [L_b] was determined directly from the titration curves using the Calvin and Melchior method [22] and the following expression

$$[L_b] = (a - a_o)\, c_{HL}$$

where a and a_o is the titration fraction of glutarimide in the presence and absence of the metal ion, respectively (at the same pH value), whereas c_{HL} corresponds to the total concentration of the protonated form of glutarimide.

The concentration of the free ligand [L] was calculated from the equation

$$[L] = K_a \frac{c_{HL} - (a - a_o)c_{HL}}{K_a + [H^+]}$$

The degree of formation, $\bar{n}$ which is the average number of ligand molecules per molecule of metal) is given by

$$\bar{n} = \frac{(a - a_o)c_{HL}}{c_M}$$

where c_M is the total concentration of metal ion.

The formation curves have been obtained from the nonlinear regression analysis and are shown in Figs. 3 and 4. The calculated stability constants of the Cu(II), Zn(II), Co(II), and Ni(II) complexes with glutarimide are given in Table 2. No stability constants for glutarimide complexes with the remaining metal ions were obtained because no shift of the corresponding titration curves was observed.

In the systems containing Cu(II), Zn(II), Co(II), and Ni(II) metal ions and glutarimide the 1 : 1 MG complexes were formed which were then followed by the 1 : 2 MG_2 species (G is the deprotonated glutarimide). It results from the data that the CuG form is present in the aqueous solution of glutarimide at the pH equal to about 3 and it is the prevailing complex

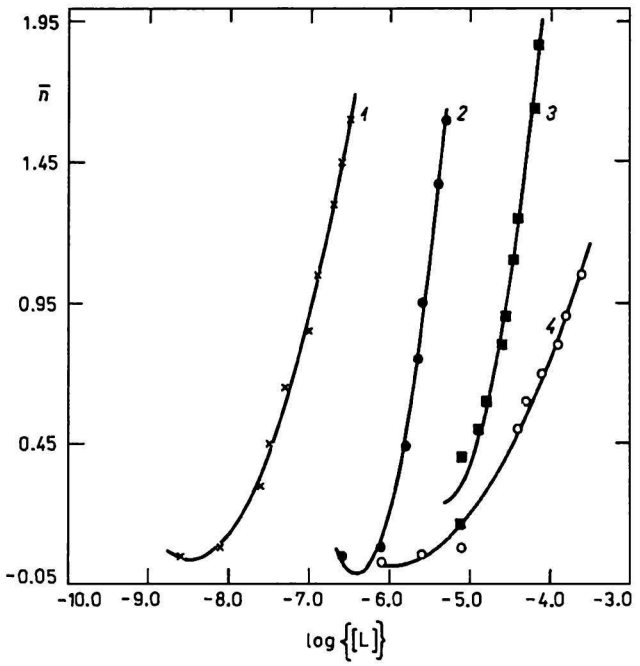

Fig. 3. Formation curves in aqueous solution. *1.* Glutarimide (G) complex with Cu(II); *2.* G with Zn(II); *3.* G with Co(II); *4.* G with Ni(II).

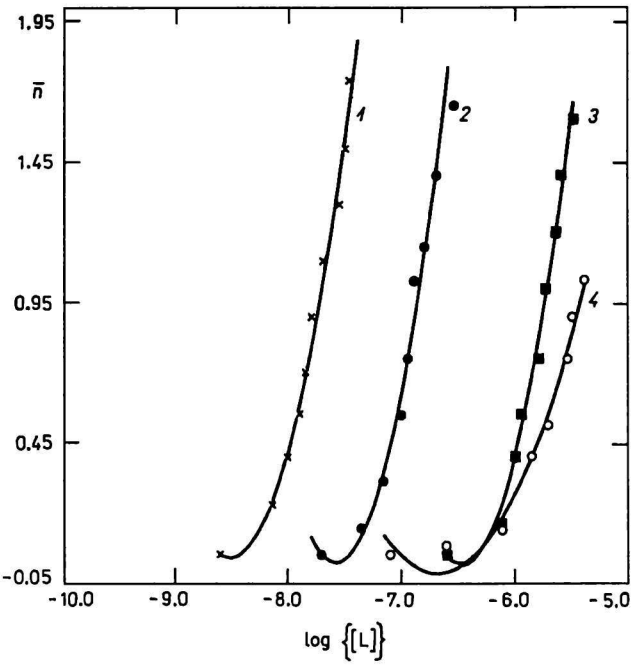

Fig. 4. Formation curves in aqueous methanol (φ = 50 vol. %). *1.* Glutarimide (G) complex with Cu(II); *2.* G with Zn(II); *3.* G with Co(II); *4.* G with Ni(II).

up to pH = 6. In the pH range between 6 and 7 the CuG_2 predominates in solution. The ZnG, CoG, and NiG forms predominate in aqueous solution up to pH equal to about 7, 8, and 8.5, respectively.

Considering stability constants, log K^{M}_{ML}, listed in Table 2, it may be concluded that of the 1 : 1 metal—glutarimide complexes the most stable is the CuG complex and the stabilities of these complexes decrease in the order Cu, Zn, Co resp. Ni. This is in accord with the Irving—Williams order of stabilities of metal complexes [23].

The 1 : 2 MG_2 complexes are less stable than the corresponding 1 : 1 MG complexes ($\Delta \log K$ is between 0.4 and 0.8), which is in a good agreement with the results reported for the metal—uridine complexes [20]. For uracil and thymine the stability constants of the complexes containing only the 1 : 1 mole ratio of the ligand to the metal ion have been obtained [21]. A comparison of the data given in Table 2 with those reported for the Cu(II), Zn(II), Co(II), and Ni(II) complexes with uridine [20] and uracil [21] indicates that glutarimide forms stronger complexes. This may be ascribed to the more basic nature of glutarimide in comparison with uridine and uracil. Stabilities of the metal complexes with glutarimide in the aqueous methanol are higher than those obtained in water. This is accompanied by a similar increase in the pK_a value of glutarimide in the water—methanol mixture.

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Resolution of DPASV Overlapping Peaks of Indium and Cadmium in Trace Analysis of High Purity Gallium Arsenide

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Computer-aided resolution of overlapping differential pulse anodic stripping voltammetry peaks of In and Cd is proposed for trace determination in high purity gallium arsenide. It is based on the fact that the signal of In appears only if the analyzed solution contains chlorides, while Cd produces signal even in the chloride-free medium. The signal of Cd obtained in the GaAs sample solution in HNO_3 is corrected to the chloride-containing medium before subtraction from the sum of unresolved peaks of In and Cd registered in the GaAs sample solution in the HNO_3—HCl mixture. The procedure was tested on ultrapure GaAs samples spiked with Cd and In. It was then applied to trace analysis of Cd and In of some industrial high purity GaAs samples.

Anodic stripping voltammetry (especially in the differential pulse mode — DPASV) on a Hg stationary electrode is commonly applied for determining impurities in high purity substances used in semiconductor components production [1]. Common metallic trace impurities of GaAs excepting In and Cd can be easily determined by this method directly in the acidic sample solution without any further adjustment since their stripping peaks are sufficiently separated. The In and Cd peak potential difference (40 mV), however, is still sufficient for their simultaneous determination by DPASV at the concentration level above 0.1 μmol dm^{-3} and with the concentration ratio close to unity. At lower concentrations and the ratio significantly differing from unity it is necessary to separate their overlapping signals.

EXPERIMENTAL

All used chemicals were of anal. grade purity. With the exception of the isothermally distilled HNO_3 they were used without any further purification. Standard solution 10^{-3} mol dm^{-3} of In^{3+} was prepared by dissolution of the corresponding amount of metal in 10 cm^3 of the concentrated acids mixture HCl—HNO_3 (φ_r = 3 : 1, heated if necessary) and dilution to 100 cm^3 with triply distilled water. Standard solution of Cd^{2+} was prepared directly from its chloride salt.

A PA 4 polarographic analyzer (Laboratorní přístroje, Prague) was used for electrochemical analysis in DPASV adjustment. Set parameters were as follows: cathodic deposition potential – 0.70 V *vs.* SCE, time 240 s; anodic stripping scan rate 10 mV s^{-1}, pulse height 12.5 mV, pulse frequency 5 Hz. Computer Compucorp 610 (Compucorp, Los Angeles, USA) equipped with A/D and D/A convertors (Burr-Brown, Tucson, USA (maximum sampling frequency 5 kHz)) on line with the electrochemical instrument was used for taking experimental curves (1000 points) and following processing of experimental data. Hanging mercury drop working electrode (HMDE) had the active surface area 1.64 mm^2.

Sample Solution Preparation

0.5 g of powdered solid GaAs was dissolved in 5 cm^3 of the mixture of concentrated acids HCl—