

Article

# Thermodynamic Solution Properties of a Biodegradable Chelant (L-glutamic-N,N-diacetic acid, L-GLDA) and Its Sequestering Ability toward Cd<sup>2+</sup>

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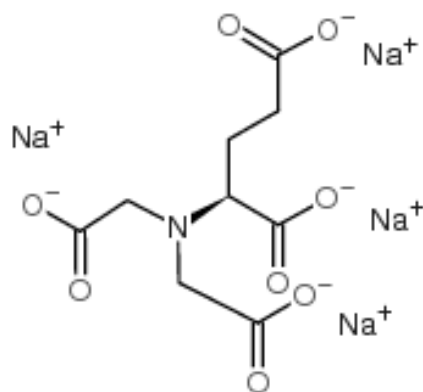
**Abstract:** The thermodynamics of the interaction of L-glutamic-N,N-diacetic acid (GLDA) with protons was studied potentiometrically at different temperatures, ionic strengths and ionic media. Four protonation constants and corresponding enthalpy changes occurred at infinite dilution together with temperature and ionic strength coefficients. The medium effect was also interpreted in terms of the formation of weak complexes between the ligand and the cations of supporting electrolytes, resulting in a greater tendency of GLDA to chemically interact with Na<sup>+</sup> rather than K<sup>+</sup> and, in turn, (CH<sub>3</sub>)<sub>4</sub>N<sup>+</sup>. Formation constants of GLDA with Cd<sup>2+</sup> were determined in NaCl<sub>(aq)</sub> at different ionic strength values. Five complex species were found, namely CdL<sup>2-</sup>, CdHL<sup>-</sup>, CdH<sub>2</sub>L<sup>0(aq)</sup>, Cd<sub>2</sub>L<sup>0(aq)</sup>, and Cd(OH)L<sup>3-</sup>, whose formation constant values at infinite dilution were log β = 12.68, 17.61, 20.76, 17.52, and 1.77, respectively. All the species results were relevant in the pH range of natural waters, although the Cd<sub>2</sub>L<sup>0(aq)</sup> was observed only for C<sub>Cd</sub> ≥ C<sub>GLDA</sub> and concentrations of >0.1 mmol dm<sup>-3</sup>. The sequestering ability of GLDA toward Cd<sup>2+</sup>, evaluated by means of pL<sub>0.5</sub>, was maximum at pH-10, whereas the presence of a chloride containing a supporting electrolyte exerted a negative effect. Among new generation biodegradable ligands, GLDA was the most efficient in Cd<sup>2+</sup> sequestration.

**Keywords:** cadmium; L-glutamic-N,N-diacetic acid; sequestering ability; potentiometry; thermodynamics; pL<sub>0.5</sub>; sustainable molecules

## 1. Introduction

The demand for biodegradable products coming from sustainable and renewable sources, for research and industrial applications, has grown considerably, with the aim to alleviate the issues related to persistent pollutants in the environment. Among chemicals widely employed in many fields of industry, chelating agents are used to efficiently control metal ions in several contexts such as household cosmetics and detergents, pharmaceuticals, the pulp and paper industry, textiles, and so forth [1–3]. Chelating agents, due to their ability to form stable complexes with metal cations, as well as their industrial applications, are responsible for various negative effects on the environment since they can mobilize radioactive metal ions or other contaminating metal cations, increasing their bioavailability, contributing to the eutrophication of water by dissolving insoluble phosphates. Most significantly, they may increase the bioavailability of dangerous heavy metals, since sometimes metal–ligand complexes such as Cu(II)-EDTA and Cd(II)-EDTA are much more toxic than free metal cations. Furthermore, the low biodegradability of the most employed chelating agents, as well as their tendency to accumulate as such in the

environment, has become an important issue. L-glutamic-N,N-diacetic acid (L-GLDA, Scheme 1) belongs to the new generation of environmentally friendly and non-toxic chelating agents [4–7]. Whereas traditional chelating agents like EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid) are negligibly biodegradable, L-GLDA was found to be readily biodegradable, according to the OECD test guidelines for chemicals [8,9]. Low toxicity ( $LD_{50} > 2000 \text{ mg kg}^{-1}$ ) makes GLDA safe for humans. GLDA can also be considered bio-based since its synthesis is based on the flavor enhancer monosodium glutamate from the fermentation of corn molasses [10].



**Scheme 1.** Structure of L-glutamic-N,N-diacetic acid (L-GLDA) tetrasodium salt.

Besides the industrial applications in a variety of formulations, GLDA was successfully tested as a potential candidate for the remediation of heavy metal contaminated soils, sewage sludge and industrial sludge, to name only a few. GLDA was investigated to assess its removal efficacy and mechanisms toward Cd, Pb and Zn from real mines and polluted farmland soils [11,12]; it was evaluated as a chelating agent for Cd, Co, Cu, Zn, Ni and Cr from sewage sludge [13]; GLDA was also found to be effective in the removal of Cd, Ni and Cu from industrial sludge [14]; it was successfully employed in the washing of soil contaminated with toxic metals, in a pilot-scale experiment to simulate real remediation operation technology [15] and as a heavy metal activator to enhance phytoremediation in red soils [16]. In addition, as a mixture with citric (CIT) or ascorbic acid, GLDA was efficiently used for Cu, as well as Pb and Zn removal from rice paddy fields, respectively [17]; chemical washing with mixed chelators, GLDA and CIT, was also successfully employed to remove heavy metals (i.e., Cd, Cu, Zn, Mn, Cr) from sewage sludge [18]; a mix of biodegradable chelators was also used in soil washing for the decontamination of different kinds of soils [19,20]. As it can be inferred from the abovementioned literature, some parameters such as the pH of the solutions employed to remove contaminants may exert a significant effect on the extraction efficacy so that strongly acidic conditions should be preferred for toxic cations removal. Among toxic metal cations, as a prioritized environmental pollutant,  $Cd^{2+}$  has received great attention since it has been recognized as harmful for humans, even at low concentrations [21–23]. Cadmium is naturally released by volcanic emissions, but anthropogenic activities greatly increase its levels in the environment (in 2020, the global refinery production of cadmium was approximately 23,000 metric tons [24], mainly from Ni–Cd battery manufacturing, nonferrous metal refining, fossil fuel combustion, incineration of municipal waste, phosphate fertilizer production, as well as electric and electronic waste recycling [25]). Cadmium contamination is a global health concern since it is easily accumulated in plants and invertebrates, affects water and soil organisms, and displays biomagnification along some food chains, thus, indirectly harming human health [26]. Moreover, cadmium distribution among soil, plants and animals is dependent on metal availability in the specific conditions occurring in the

environment so that its removal is largely related to its chemical speciation. In this light, the assessment of the speciation models of  $\text{Cd}^{2+}$  in the presence of GLDA is crucial since it can be a robust support in predicting the complex formation in specific experimental conditions. Moreover, the evaluation of species formation and relative stability, as well as the determination of the chelation thermodynamics of GLDA toward  $\text{Cd}^{2+}$ , is essential to specifically design a sequestration strategy in view of practical heavy metal decontamination. Accordingly, a systematic study on the thermodynamics of the interaction of GLDA with protons, reporting data in a standard state (i.e., infinite dilution) and parameters to calculate protonation constants in different ionic media ( $\text{NaCl}$ ,  $\text{KCl}$ ,  $(\text{CH}_3)_4\text{NCl}$ , and  $(\text{C}_2\text{H}_5)_4\text{NI}$ ) at different temperatures and ionic strengths, is presented here. Moreover, the formation constants and sequestering ability of GLDA toward  $\text{Cd}^{2+}$  are determined in a wide range of experimental conditions and compared to data reported for other “traditional” and “new generation” chelating agents.

## 2. Materials and Methods

### 2.1. Chemicals

Tetraethylammonium iodide  $[(\text{C}_2\text{H}_5)_4\text{NI}]$  was re-crystallized from methanol as described by Perrin et al. [27]. Other chemicals were purchased from Merck Italy at the highest purity available and were used without further purification. High purity L- glutamic-N,N-diacetic acid tetrasodium salt (GLDA, Dissolvine® GL-PD-S), was obtained from AkzoNobel (Amsterdam) and was used without further purification. Potassium hydroxide, sodium hydroxide, tetraethylammonium hydroxide and hydrochloric acid solutions were prepared from concentrated solutions and standardized against potassium hydrogen phthalate (for bases) and sodium carbonate (for acids), previously dried in the oven at 383.15 K for 2 h. The concentration of GLDA in the solutions was determined by alkalimetric titrations. Sodium and potassium chloride solutions were prepared by weighing the solid previously dried in the oven at 383.15 for 2 h. Cadmium chloride solutions were prepared by weighing the salt and then standardized against EDTA. All solutions were freshly prepared, using grade A glassware and twice-distilled water ( $\rho \geq 18 \text{ M}\Omega$ ). All chemicals used are listed in Table 1.

**Table 1.** Chemicals used in this work, purchased from Sigma-Aldrich. Purity (mass) as stated by the supplier.

Chemical	Formula	CAS n°	Purification	Assay (Mass)
Sodium chloride	$\text{NaCl}$	7647-14-5	NO	$\geq 99\%$
Potassium chloride	$\text{KCl}$	7447-40-7	NO	$\geq 99\%$
Tetraethylammonium iodide	$(\text{C}_2\text{H}_5)_4\text{NI}$	68-05-3	[27]	98%
Hydrochloric acid	$\text{HCl}$	7647-01-0	NO	$\geq 99\%$
Tetraethylammonium hydroxide	$(\text{C}_2\text{H}_5)_4\text{NOH}$	77-98-5	NO	$\sim 10\%$ <sup>a</sup>
Sodium hydroxide	$\text{NaOH}$	1310-73-2	NO	$\geq 99\%$
Potassium phthalate monobasic	$\text{C}_8\text{H}_5\text{O}_4\text{K}$	877-24-7	NO	$\geq 99.95\%$
Sodium carbonate	$\text{Na}_2\text{CO}_3$	497-19-8	NO	99.995%
Cadmium chloride hydrate	$\text{CdCl}_2 \cdot x \text{H}_2\text{O}$	654054-66-7	NO	$\geq 98\%$
L-glutamic acid N,N-diacetic acid tetrasodium salt (GLDA)	$\text{C}_9\text{H}_{10}\text{N}_1\text{O}_8\text{Na}_4$	51981-21-6	NO	$> 80\%$ <sup>b</sup>

<sup>a</sup> The value refers to the concentration in the solutions. On the dry basis, the assay is  $\geq 0.995$  (on the mass basis); <sup>b</sup> AkzoNobel declares the product is high purity and NTA free ( $< 0.2\%$ ).

## 2.2. Apparatus and Procedure for the Potentiometric Measurements

The acid–base properties of GLDA and its interaction with  $\text{Cd}^{2+}$  were studied by ISE- $\text{H}^+$  potentiometric titrations carried out using a Metrohm 809 Titrand equipped with an automatic burette (total volume  $10 \text{ cm}^3$ ) and a combined glass electrode (Metrohm, model 6.0262.100). The estimated accuracy for both systems was  $\pm 0.2 \text{ mV}$  and  $\pm 0.003 \text{ mL}$  for e.m.f. and titrant volume readings, respectively. The apparatuses were connected to a PC and controlled by Tiamo 2.5 software to set titrant delivery, data acquisition and to check for e.m.f. stability. All titrations were carried out under magnetic stirring; presaturated  $\text{N}_2$  was bubbled through the solution to exclude/prevent  $\text{O}_2$  and  $\text{CO}_2$  dissolution. For each measurement  $25 \text{ cm}^3$  of titrand solution containing a suitable amount of GLDA ( $1.0 \leq C_L/\text{mmol dm}^{-3} \leq 5.0$ ),  $\text{HCl}_{(\text{aq})}$  and ionic medium ( $\text{NaCl}_{(\text{aq})}$ ,  $\text{KCl}_{(\text{aq})}$  or  $(\text{C}_2\text{H}_5)_4\text{NI}_{(\text{aq})}$ ) was added to reach pre-established the values of pH ( $\sim 2.0$ ) and ionic strengths ( $0.1 \leq I/\text{mol dm}^{-3} \leq 1.0$ ). For the measurements aiming to determine the stability constants of the  $\text{Cd}^{2+}/\text{GLDA}$  system,  $\text{CdCl}_2$  ( $0.4 \leq C_M/\text{mmol dm}^{-3} \leq 2.0$ , generally,  $C_M \leq C_L$ ) was added into the cell. The titrand solutions were titrated with  $\text{CO}_2$ -free standard base ( $\text{NaOH}_{(\text{aq})}$ ,  $\text{KOH}_{(\text{aq})}$  or  $(\text{C}_2\text{H}_5)_4\text{NOH}_{(\text{aq})}$ ) solutions up to pH  $\sim 10.0$  or until the electrode started drifting toward lower pH values which indicated the onset of precipitation of sparingly soluble species hardly detectable by the naked eye in the initial state. Potentiometric measurements were performed at different temperatures whose value was constantly maintained by using circulating water from a thermocryostat (model D1-G Haake). Further details on the potentiometric measurements are reported in Table 2.

**Table 2.** Summary of the potentiometric titrations performed (L stands for GLDA) performed in different ionic media at different temperatures, ionic strengths, and  $p = 0.1 \text{ MPa}$ .

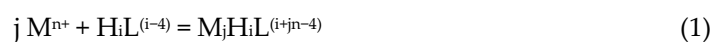
System	Medium	T/K	$I^a$	$C_H^b$	$C_L^b$	$C_M^b$	n.tit
$\text{H}^+/\text{L}$	NaCl	288.15 and 310.15	0.1 to 1.0	10 to 20	2.5 to 4		24
$\text{H}^+/\text{L}$	KCl	288.15 to 310.15	0.1 to 1.0	10 to 18	2.5 to 4		33
$\text{H}^+/\text{L}$	$(\text{C}_2\text{H}_5)_4\text{NI}$	288.15 to 310.15	0.1 to 1.0	10 to 20	3.0 to 5		29
$\text{Cd}^{2+}/\text{L}$	NaCl	298.15	0.1 to 1.0	8.0 to 10	1.0 to 4.0	0.4 to 2.0	30

<sup>a</sup>  $\text{mol dm}^{-3}$ ; <sup>b</sup>  $\text{mmol dm}^{-3}$ ; standard uncertainties ( $u$ ):  $u(T) = 0.1 \text{ K}$ ;  $u(I) = 0.001 \text{ mol dm}^{-3}$ ;  $u(p) = 1 \text{ kPa}$ ;  $u(C_H) = u(C_L) = u(C_M) = 10^{-2} \text{ mmol dm}^{-3}$ .

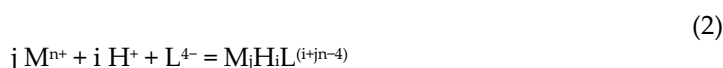
## 2.3. Calculations

ESAB2M [28] was used to refine all the parameters of the acid–base potentiometric titrations ( $E^0$ ,  $K_w$ , liquid junction potential coefficient,  $j_a$  and analytical concentration of reagents) whereas BSTAC [29] was employed for the calculation of equilibrium constants. The non-linear least square computer program LIANA was used to fit different equations [29]. ES2WC was used for the calculation of the pure model approach [30].

All equilibria, stepwise ( $K_{ij}$ ) and overall ( $\beta_{ij}$ ) described in this paper are expressed by the following Equations:



or



where M, H and L are the metal ion of interest, the proton and the GLDA anion ( $\text{Glda}^{4-}$ ), respectively. Distribution diagrams were obtained by using HySS [31]. Throughout the paper, uncertainties are given as a 95 % confidence interval. The conversion from the molar to the molal concentration scale was performed using the appropriate density values [32].

The ionic strength dependence of the equilibrium constants was studied in this work by means of the extended Debye–Hückel equation (EDH) and the Specific ion Interaction Theory (SIT) [33–37] model. Considering a generic complex formation constant, expressed

in Equation (2), the equilibrium constants, as a function of the activity coefficients, may be expressed by Equation (3):

$$\log \beta_{ij} = \log \beta_{ij}^0 + i \cdot \log \gamma_{H^+} + j \cdot \log \gamma_{M^{n+}} + \log \gamma_{L^4} - \log \gamma_{M_j H_i L^{(i+jn-4)}} \quad (3)$$

where  $\beta_{ij}$  and  $\beta_{ij}^0$  are the stoichiometric (or conditional) and thermodynamic equilibrium constant, respectively. Both EDH and SIT models assume that the variation of the activity coefficients ( $\gamma$ ) with charge ( $z$ ) and ionic strength ( $I$ ) can be expressed by the general formula:

$$\log \gamma = -z^2 \cdot A \cdot I^{0.5} / (1 + 1.5 \cdot I^{0.5}) + f(I) \quad (4)$$

$$A = \left( 0.51 + \frac{0.856 \cdot (T - 298.15) + 0.00385 \cdot (T - 298.15)^2}{1000} \right) \quad (5)$$

where  $f(I)$  is a term accounting for ionic strength dependence. Rearranging Equation (3) according to Equation (4) leads to:

$$\log \beta_{ij} = \log \beta_{ij}^0 - 0.51 \cdot z^* \cdot \text{D.H.} + f(I) \quad (6)$$

$$\text{D.H.} = \frac{\sqrt{I}}{1 + 1.5 \cdot \sqrt{I}} \quad (7)$$

$$z^* = \sum (\text{charge})^2_{\text{reactant}} - \sum (\text{charge})^2_{\text{product}} \quad (8)$$

Equation (5) comes from the polynomial fitting of tabulated values of  $A$  at different temperatures.

The differences between the two models stem from the nature of the  $f(I)$  term as well as from the adopted concentration scale, namely molar for the EDH equation, Equation (9), and molal the SIT model, Equation (10):

$$\log \beta_{ij} = \log \beta_{ij}^0 - A \cdot z^* \cdot \text{D.H.} + C_{ij} \cdot I_c \quad (9)$$

$$\log \beta_{ij} = \log \beta_{ij}^0 - A \cdot z^* \cdot \text{D.H.} + \Delta \varepsilon_{ij} \cdot I_m \quad (10)$$

In the SIT model,  $\Delta \varepsilon_{ij}$  is the combination of the specific interaction coefficients of the species involved in the equilibrium and the ions of the supporting electrolyte. For example, in the case of Equation (3) in  $\text{NaCl}_{(\text{aq})}$ , it is:

$$\Delta \varepsilon_{ij} = i \cdot \varepsilon(\text{H}^+, \text{Cl}^-) + j \cdot \varepsilon(\text{M}^{n+}, \text{Cl}^-) + \varepsilon(\text{Na}^+, \text{L}^{4-}) - \varepsilon(\text{Na}^+, \text{M}_j \text{H}_i \text{L}^{(i+jn-4)}) \quad (11)$$

When a neutral species, such as the  $\text{M}_2\text{L}$  species for a divalent metal cation, is involved, Equation (11) becomes:

$$\Delta \varepsilon_{02} = 2 \cdot \varepsilon(\text{M}^{2+}, \text{Cl}^-) + \varepsilon(\text{Na}^+, \text{L}^{4-}) - k_m(\text{M}_2\text{L}) \quad (12)$$

where  $k_m$  is the Setschenow coefficient.

The dependence of the equilibrium constant on temperature was modeled by the van't Hoff equation, as follows:

$$\log \beta_{ij} = \log \beta_{ij \theta} + \frac{\Delta H_{ij}}{R \cdot \ln(10)} \cdot \left( \frac{1}{\theta} - \frac{1}{T} \right) \quad (13)$$

where  $\theta$  is the reference temperature ( $\theta = 298.15$  K in our case) and  $\Delta H_{ij}$  is the enthalpy variation of the equilibrium, whose dependence on ionic strength is given by:

$$\Delta H_{ij} = \Delta H_{ij}^0 - z^* \cdot A' \cdot \frac{\sqrt{I}}{1 + 1.5 \cdot \sqrt{I}} + \Delta \varepsilon'_{ij} \cdot I \quad (14)$$

where  $\Delta H_{ij}^0$  is the enthalpy variation of the equilibrium at infinite dilution, and  $\Delta \epsilon'_{ij}$  is the ionic strength dependence parameters of  $\Delta H_{ij}^0$ ;  $A'$  and  $\Delta \epsilon'_{ij}$  are related to  $A$  and  $\Delta \epsilon_{ij}$  as follows:

$$A' = RT^2 \cdot \ln(10) \cdot \frac{\partial A}{\partial T} = 1.5 + 0.024 \cdot (T - 298.15) \quad (15)$$

$$\Delta \epsilon'_{ij} = RT^2 \cdot \ln(10) \cdot \frac{\partial \Delta \epsilon_{ij}}{\partial T} \quad (16)$$

Combining Equation (10) with Equation (14), the equation to which the experimental data were fitted is:

$$\log \beta_{ij} = \log \beta_{ij\theta}^0 - z^* \cdot A \cdot \frac{\sqrt{I}}{1 + 1.5 \cdot \sqrt{I}} + \Delta \epsilon_{ij} \cdot I + \frac{\Delta H_{ij}}{R \cdot \ln(10)} \cdot \left( \frac{1}{\theta} - \frac{1}{T} \right) \quad (17)$$

Conventionally, thermodynamic parameters refer to the molal concentration scale, and the symbolism referred to in the molar concentration scale has the superscript “M” (e.g., the protonation enthalpy change at infinite dilution in the molar concentration scale is indicated by  $\Delta H_{i0}^{0M}$ ).

The sequestering ability of GLDA toward  $\text{Cd}^{2+}$  is computed by calculating  $\text{pL}_{0.5}$ . This semi-empirical parameter allows comparisons among ligands as it eliminates the contribution of ligand protonation constants, metal cation hydrolysis constants and any other reaction (e.g., chloride complexes) competing with it under analysis, namely the formation constants of the M/L system. Moreover,  $\text{pL}_{0.5}$  does not depend on the speciation scheme, but only on the experimental conditions (pH, ionic strength, and temperature) used for its computation.

Briefly, the higher the  $\text{pL}_{0.5}$  is the stronger the sequestering ability. The reader may find further details concerning  $\text{pL}_{0.5}$  definition and use in [38–42].

### 3. Results and Discussion

For reasons of transparency and to report the real experimental data of this study, the data couples represented by the  $\text{cm}^3$  of titrant vs. e.m.f. obtained for all the potentiometric titrations analyzed in this work are reported as Supplementary Materials (Excel spreadsheet). The pairs of values represent all the recorded points, but not all the recorded points have necessarily been taken into consideration during the data analysis, as different pH values were explored.

#### 3.1. Acid–Base Properties of L-glutamic-N,N-diacetic Acid

The acid–base properties of the L-glutamic-N,N-diacetic acid were obtained in the molar concentration scale at different ionic strengths, temperatures and various ionic media. Conditional protonation constant values were then converted to a molal concentration scale as reported in the experimental section. As the amount of data is too large, the full record of the values obtained, both in the molar and in the molal concentration scales, is reported as Supplementary Materials (Table S1).

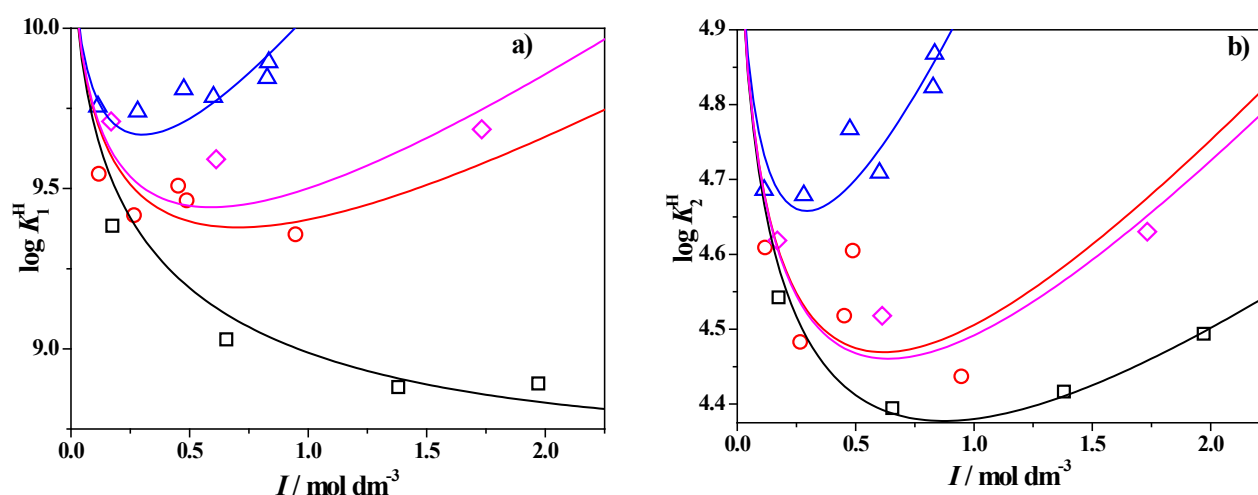
The data analysis, according to all models mentioned, was performed using the protonation constants reported in this work at different temperatures and ionic strengths in aqueous NaCl, KCl, and  $(\text{C}_2\text{H}_5)_4\text{NI}$  solutions and those already published by Bretti et al. [43] at 298.15 K in aqueous NaCl,  $(\text{CH}_3)_4\text{NCl}$  and  $(\text{C}_2\text{H}_5)_4\text{NI}$  at  $I \leq 2.134 \text{ mol kg}^{-1}$ . This last choice was required to fit protonation data as in Equation (9), with the consideration that the term  $f(I)$  is linear, whereas Bretti et al. [43] used a two-parameter approach.

The trend observed for the protonation constants values, especially regarding the HL and  $\text{H}_2\text{L}$  species, is reported in Figure 1 and it traces what has already been discussed by Bretti et al. [43] as values in  $(\text{C}_2\text{H}_5)_4\text{NI}$  are higher than those in  $(\text{CH}_3)_4\text{NCl}$ , which in turn, is higher than KCl and NaCl. This behavior indicates that sodium cation can interact with

the fully deprotonated and the monoprotinated GLDA anion more when compared to the potassium cation and tetramethylammonium, whereas tetraethylammonium seems to be the cation less able to interact among those used as the background electrolyte. The continuous line depicted in Figure 1 represents the fitting to the EDH model.

For the  $H_3L$  and  $H_4L$  species, whose values are much lower compared to the first two protonation constants, the trend is less evident.

The trend discussed is well-established regarding  $\log K^{H_1}$  with relevant differences among ionic media, i.e., the whole stability constant values are spread within one order of magnitude in terms of the protonation constant. Contrarily, differences among  $\log K^{H_2}$  (Figure 1b) are less important and in some cases, such as in the comparison between KCl and  $(CH_3)_4NCl$ , are within the experimental errors.



**Figure 1.** Dependence of the first (a) and second (b) protonation constants of GLDA on ionic strength at  $T = 298.15$  K in  $NaCl_{aq}$  ( $\square$ ),  $KCl_{aq}$  ( $\circ$ ),  $(CH_3)_4NCl_{aq}$  ( $\diamond$ ) and  $(C_2H_5)_4NCl_{aq}$  ( $\Delta$ ).

Once the protonation constants, both in the molar and in the molal concentration scales are provided, the data analysis was performed with the EDH, SIT and pure water model approaches. Regarding the EDH and SIT approaches, the whole set of conditional protonation constants (Table S2) was fitted to Equation (17) and a set of parameters useful for calculating the protonation constants of GLDA at any value of temperature and ionic strength within the experimental domain was obtained (Table 3). During this fitting procedure, the values of the protonation constants at infinite dilution and  $T = \theta = 298.15$  K ( $\log \beta_{10\theta}^0$ ) were fixed in accordance with those already published by Bretti et al. [43].

The parameters reported in Table 3 show some trends, for example, the values of  $C_i$  within the same ionic medium decrease with increasing the number of protons of the species. The only exception to this trend is represented by the  $C_1$  reported for  $NaCl_{aq}$ .

Regarding the values of protonation enthalpy changes, the value found for the first step, namely  $\Delta H_{1\theta}^0 = (-11.6 \pm 1.4)$  kJ mol $^{-1}$ , is slightly less negative compared to other values reported in the literature for the interaction of a proton with a ternary amine; for example,  $\Delta H_{1\theta}^0 = -25$  kJ mol $^{-1}$  for methylglycindiactic acid [44],  $\Delta H_{2\theta}^0 = -19$  kJ mol $^{-1}$  for risedronic acid [45],  $\Delta H_{1\theta}^0 = -23$  kJ mol $^{-1}$  for ethylenediaminetetracetic acid [46] and  $\Delta H_{1\theta}^0 = -19$  kJ mol $^{-1}$  for nitrilotriacetic acid was found [46]. Compared to the parent glutamic acid molecule [47], the protonation constant at infinite dilution of  $Glda^{4-}$  is slightly higher ( $\log K_{10\theta}^0 = 10.56$  and  $10.01$ , for  $Glda^{4-}$  and glutamate, respectively), whereas the protonation enthalpy is significantly lower ( $\Delta H_{10\theta}^{0M} = -12$  and  $-40$ , same order), resulting in different thermodynamic behavior for proton binding, being enthalpically-driven for glutamate and entropically-driven for  $Glda^{4-}$ .

**Table 3.** Parameters obtained fitting experimental protonation constants to Equation (17) in the molar concentration scales at  $T = 298.15$  K and  $p = 0.1$  MPa.

Molar Concentration Scale										
			NaCl		KCl		(CH <sub>3</sub> ) <sub>4</sub> NCl		(C <sub>2</sub> H <sub>5</sub> ) <sub>4</sub> NI	
i	log $K_{i0\theta}^0$	$\Delta H_{i0\theta}^{0M}$	$z^*$ <sup>a</sup>	$C_{i0}$	$\Delta \epsilon_{i0}^{M}$	$C_{i0}$	$\Delta \epsilon_{i0}^{M}$	$C_{i0}$	$C_{i0}$	$\Delta \epsilon_{i0}^{M}$
1	10.560	$-11.6 \pm 1.4$ <sup>b</sup>	8	$-0.03 \pm 0.01$ <sup>b</sup>	$8 \pm 5$ <sup>b</sup>	$0.46 \pm 0.02$ <sup>b</sup>	$0 \pm 3$ <sup>b</sup>	$0.55 \pm 0.03$ <sup>b</sup>	$1.07 \pm 0.02$ <sup>b</sup>	$-4 \pm 3$ <sup>b</sup>
2	5.324	$2.0 \pm 0.9$	6	$0.28 \pm 0.01$	$-20 \pm 3$	$0.34 \pm 0.02$	$2 \pm 1$	$0.37 \pm 0.02$	$0.82 \pm 0.01$	$0 \pm 2$
3	3.071	$3.0 \pm 1.9$	4	$0.16 \pm 0.02$	$-13 \pm 2$	$0.16 \pm 0.02$	$1 \pm 1$	$0.27 \pm 0.02$	$0.63 \pm 0.02$	$0 \pm 1$
4	1.897	$-7.0 \pm 3.5$	2	$0.09 \pm 0.02$	$-6 \pm 1$	$0.02 \pm 0.09$	$0 \pm 1$	$0.25 \pm 0.01$	$0.55 \pm 0.07$	$0 \pm 1$

Molal Concentration Scale										
			NaCl		KCl		(CH <sub>3</sub> ) <sub>4</sub> NCl		(C <sub>2</sub> H <sub>5</sub> ) <sub>4</sub> NI	
i	log $K_{i0\theta}^0$	$\Delta H_{i0\theta}^0$	$z^*$ <sup>a</sup>	$\Delta \epsilon_{i0}$	$\Delta \epsilon_{i0}^{\prime}$	$\Delta \epsilon_{i0}$	$\Delta \epsilon_{i0}^{\prime}$	$\Delta \epsilon_{i0}$	$\Delta \epsilon_{i0}$	$\Delta \epsilon_{i0}^{\prime}$
1	10.559	$-12.0 \pm 1.4$ <sup>b</sup>	8	$-0.03 \pm 0.01$ <sup>b</sup>	$8 \pm 5$ <sup>b</sup>	$0.45 \pm 0.02$ <sup>b</sup>	$0 \pm 3$ <sup>b</sup>	$0.44 \pm 0.03$ <sup>b</sup>	$0.91 \pm 0.02$ <sup>b</sup>	$-4 \pm 3$ <sup>b</sup>
2	5.323	$1.7 \pm 0.9$	6	$0.27 \pm 0.01$	$-20 \pm 3$	$0.33 \pm 0.02$	$2 \pm 1$	$0.29 \pm 0.02$	$0.69 \pm 0.01$	$-1 \pm 2$
3	3.070	$3.0 \pm 1.9$	4	$0.15 \pm 0.02$	$-13 \pm 2$	$0.15 \pm 0.02$	$1 \pm 1$	$0.19 \pm 0.02$	$0.49 \pm 0.02$	$-1 \pm 1$
4	1.896	$-6.9 \pm 3.5$	2	$0.08 \pm 0.02$	$-7 \pm 1$	$0.01 \pm 0.09$	$1 \pm 1$	$0.16 \pm 0.01$	$0.44 \pm 0.07$	$0 \pm 1$

<sup>a</sup>  $z^*$  is a parameter of Equation (17); <sup>b</sup>  $\pm s$  (standard deviation); standard uncertainties ( $u$ ):  $u(T) = 0.1$  K;  $u(p) = 1$  kPa;  $u(\log \beta_{i0\theta}^0) = 0.005$ .

Using the data listed in Table 3 in the molal concentration scale, thermodynamic parameters ( $\Delta G^0$ ,  $\Delta H^0$ , and  $T\Delta S^0$ , in kJ mol<sup>-1</sup>) were calculated at different ionic strength values. The data given in Table 4 reveal that the first (relative to the amino group) and the fourth protonation steps are exothermic in all conditions; the second and the third steps are slightly endothermic at low ionic strength values and tend to become less positive at  $I \geq 0.25$  mol kg<sup>-1</sup> or negative in the case of sodium chloride. The contribution to the proton binding is generally entropic in nature for the first, second and third protonation steps in all ionic media and at all ionic strength values. Conversely, the fourth protonation step is enthalpic in nature with this trend even more evident at  $I \geq 0.75$  mol kg<sup>-1</sup> in NaCl<sub>aq</sub>. Similar behaviors were observed in the literature for molecules with the same functional groups (e.g., [38,44]).

**Table 4.** Thermodynamic parameters ( $\Delta G^0$ ,  $\Delta H^0$  and  $T\Delta S^0$ , in kJ mol<sup>-1</sup>) for the four GLDA protonation steps (i) at different ionic strength values and  $T = 298.15$  K and  $p = 0.1$  MPa.

		NaCl			KCl			(C <sub>2</sub> H <sub>5</sub> ) <sub>4</sub> NI			
$T$	$I$ <sup>a</sup>	i	$-\Delta G^0$	$\Delta H^0$	$T\Delta S^0$	$-\Delta G^0$	$\Delta H^0$	$T\Delta S^0$	$-\Delta G^0$	$\Delta H^0$	$T\Delta S^0$
298.15	0.00	1	60.3	-12	48	60.3	-12	48	60.3	-12	48
298.15	0.00	2	30.4	2	32	30.4	2	32	30.4	2	32
298.15	0.00	3	17.5	3	21	17.5	3	21	17.5	3	21
298.15	0.00	4	10.8	-7	4	10.8	-7	4	10.8	-7	4
298.15	0.10	1	55.3	-14	41	55.5	-15	41	55.8	-15	41
298.15	0.10	2	26.8	-2	25	26.8	0	27	27.0	0	27
298.15	0.10	3	15.1	0	16	15.1	2	17	15.3	2	17
298.15	0.10	4	9.6	-8	2	9.6	-7	2	9.8	-7	2
298.15	0.25	1	53.6	-13	40	54.3	-15	39	54.9	-16	38
298.15	0.25	2	25.8	-6	20	25.9	0	25	26.4	-1	25
298.15	0.25	3	14.4	-2	12	14.4	2	16	14.9	1	16
298.15	0.25	4	9.3	-9	0	9.2	-7	2	9.8	-8	2
298.15	0.50	1	52.2	-12	40	53.6	-16	37	54.9	-18	37
298.15	0.50	2	25.2	-11	14	25.3	0	25	26.4	-2	24
298.15	0.50	3	14.0	-6	8	14.0	1	15	14.9	0	15
298.15	0.50	4	9.1	-11	-2	8.9	-7	2	10.1	-8	2

298.15	0.75	1	51.4	-11	41	53.4	-17	37	55.4	-20	36
298.15	0.75	2	25.0	-17	8	25.2	0	25	26.8	-2	24
298.15	0.75	3	13.8	-9	5	13.8	1	15	15.2	0	15
298.15	0.75	4	9.0	-13	-4	8.7	-7	2	10.5	-8	3
298.15	1.00	1	50.8	-9	42	53.5	-17	37	56.1	-21	35
298.15	1.00	2	24.9	-22	3	25.3	0	25	27.3	-3	24
298.15	1.00	3	13.7	-12	1	13.7	2	15	15.7	0	15
298.15	1.00	4	9.0	-15	-6	8.6	-7	2	11.0	-8	3

<sup>a</sup> in mol kg<sup>-1</sup>; standard uncertainties (*u*): *u*(*T*) = 0.1 K; *u*(*I*) = 0.01 mol kg<sup>-1</sup>; *u*(*p*) = 1 kPa; *u*(Δ*G*<sup>0</sup>) = 0.2 kJ mol<sup>-1</sup>; *u*(Δ*H*<sup>0</sup>) = 1 kJ mol<sup>-1</sup>; *u*(*T*Δ*S*<sup>0</sup>) = 1 kJ mol<sup>-1</sup>.

Experimental protonation constants (Table S2) were also analyzed by the “pure water model”. This approach is based on a chemical model, in which the different ionic strength dependence of conditional protonation constants observed in different ionic media are dependent on the chemical interaction taking place between the ligand (L) and the ions of the supporting electrolyte (e.g., NaCl), through the formation of several “weak species”, such as NaL. The aim of this approach is to split the conditional protonation constants to obtain “pure” protonation constants valid in any ionic media and weak species relative to the specific cation or anion interacting with the ligand. The ionic strength dependence of both protonation constants and weak species is a function only of the reaction stoichiometry within a reasonably narrow ionic strength range ( $0 < I/\text{mol L}^{-1} \leq 1.0$ ). The chemical speciation of a ligand in a multi-component solution must be drawn considering the “pure” protonation constants and the weak species relative to all the components of the solution calculated at the ionic strength of such solution and considering the concentration of all the interacting ions. Theoretical bases about this approach are available in the literature, including the requirements of using the molar concentration scale, both for ionic strength and equilibrium constants [48,49], the presence of a background electrolyte not—or very weakly—interacting with the ligand (generally tetraethylammonium iodide), and the ionic strength range not exceeding  $I = 1.0 \text{ mol dm}^{-3}$  [50–53]. Some successful examples of applications of this model to other molecules are reported in the literature (e.g., [44,45,54]), where the readers may also find extensive discussion about the fitting ability and the analogy with the hybrid SIT model in terms of the chemical information carried.

As already mentioned, according to the pure water model the ionic strength dependence of the equilibrium constants is just a function of the stoichiometric coefficients and it is described by the following Equation:

$$\log K = \log K^0 - z^* \cdot (I^{0.5}/(2 + 3 \cdot I^{0.5})) + I \cdot (c_0 \cdot p^* + c_1 \cdot z^*) - 0.1 \cdot z^* \cdot I^{1.5} \quad (18)$$

$$p^* = \sum (\text{stoich. coeff})_{\text{reactants}} - \sum (\text{stoich. coeff})_{\text{products}} \quad (19)$$

where  $\log K$  can be the protonation constant ( $K^{\text{H}}$ ) or the weak complex formation constant ( $K_{\text{MjHL}}$ ),  $\log K^0$  is the same quantity at infinite dilution,  $c_0$  and  $c_1$  are the ionic strength dependence parameters common to all the species, and  $z^*$  is given in Equation (8).

Whenever data at different temperatures are available, adjustable parameters of Equation (18) (i.e.,  $\log K^0$ ,  $c_0$ ,  $c_1$ ) can be written as follows:

$$Y_T = Y_\theta + \frac{\partial Y}{\partial T} \cdot (T - \theta) \quad (20)$$

$$\frac{\partial \log K}{\partial T} = \frac{\Delta H_{10}^{\text{0M}}}{RT^2 \ln(10)} \approx \frac{\Delta H_{10}^{\text{0M}}}{1702} \quad (\text{at } T = 298.15 \text{ K}) \quad (21)$$

where  $Y_T$  is the value of the parameters at the temperature  $T$ ,  $Y_\theta$  is the value at the reference temperature  $\theta$  (298.15 K) and the partial derivative accounts for the temperature dependence. The first derivative parameter originates from the expansion to the Taylor series of the parameter  $Y$  approximately at the point  $\theta$ . For  $Y = \log K$  this is the first derivative

on the temperature of Equation (13), which in turn corresponds to  $\Delta H_{i0\theta}^{0M}$  divided by  $RT^2 \ln(10)$  (Equation (21)).

To avoid misinterpretations, protonation constants at infinite dilution were kept fixed to values reported in Table 3 (in the molar concentration scale) and the value of the partial derivative accounting for the temperature dependence of  $\log K$  was obtained dividing the value of  $\Delta H_{i0\theta}^{0M}$  reported in Table 3 by the value of 1702 (Equation (21)). Moreover, to bypass the correlation between  $c_0$  and  $c_1$  parameters of Equation (18) during the fitting procedure, it was chosen to refine only one of them. Since values of  $z^*$  are higher than those of  $p^*$  (see Table 5), it was preferred to refine  $c_1$  and to fix the value of  $c_0$  at  $c_0 = 0.165$ .

The conditional protonation data in the molar concentration scale at different ionic strengths and temperatures and in different ionic media were analyzed with ES2WC software [30], the best fit was obtained by refining the equilibrium constants of thirteen weak species, namely NaL, Na<sub>2</sub>L, NaHL, NaH<sub>2</sub>L, NaH<sub>3</sub>L, KL, KHL, KH<sub>2</sub>L, KH<sub>3</sub>L, (CH<sub>3</sub>)<sub>4</sub>NL, (CH<sub>3</sub>)<sub>4</sub>NHL, (CH<sub>3</sub>)<sub>4</sub>NH<sub>2</sub>L and (CH<sub>3</sub>)<sub>4</sub>NH<sub>3</sub>L. A summary of all the parameters refined is given in Table 5.

**Table 5.** Equilibrium constants at infinite dilution, temperature and ionic strength dependence parameters of the weak species for GLDA (L) at  $T = 298.15$  K and  $p = 0.1$  MPa.

Equilibrium	$p^*$ <sup>a</sup>	$z^*$ <sup>a</sup>	$Y_\theta$ ( $\log \beta^0$ )	$\frac{\partial Y}{\partial T}$
$H^+ + L^{4-} = HL^{3-}$	1	8	10.56 <sup>b</sup>	-0.0068 <sup>b</sup>
$2 H^+ + L^{4-} = H_2L^{2-}$	2	14	15.88 <sup>b</sup>	-0.0056 <sup>b</sup>
$3 H^+ + L^{4-} = H_3L^-$	3	18	18.96 <sup>b</sup>	-0.0039 <sup>b</sup>
$4 H^+ + L^{4-} = H_4L^{0(aq)}$	4	20	20.86 <sup>b</sup>	-0.008 <sup>b</sup>
$Na^+ + L^{4-} = NaL^{3-}$	1	8	$2.50 \pm 0.02$ <sup>c</sup>	$0.017 \pm 0.004$ <sup>c</sup>
$Na^+ + H^+ + L^{4-} = NaHL^{2-}$	2	14	$12.25 \pm 0.03$	$0.013 \pm 0.004$
$Na^+ + 2 H^+ + L^{4-} = NaH_2L^-$	3	18	$16.83 \pm 0.03$	$0.007 \pm 0.003$
$Na^+ + 3 H^+ + L^{4-} = NaH_3L^{0(aq)}$	4	20	$19.08 \pm 0.07$	$0.003 \pm 0.007$
$2 Na^+ + L^{4-} = Na_2L^{2-}$	2	14	$3.01 \pm 0.10$	$0.018 \pm 0.012$
$K^+ + L^{4-} = KL^{3-}$	1	8	$2.20 \pm 0.05$	$0.004 \pm 0.005$
$K^+ + H^+ + L^{4-} = KHL^{2-}$	2	14	$12.12 \pm 0.04$	$-0.004 \pm 0.004$
$K^+ + 2 H^+ + L^{4-} = KH_2L^-$	3	18	$16.81 \pm 0.03$	$-0.004 \pm 0.004$
$K^+ + 3 H^+ + L^{4-} = KH_3L^{0(aq)}$	4	20	$19.11 \pm 0.06$	$-0.003 \pm 0.007$
$(CH_3)_4N^+ + L^{4-} = (CH_3)_4NL^{3-}$	1	8	$1.89 \pm 0.08$	-
$(CH_3)_4N^+ + H^+ + L^{4-} = (CH_3)_4NHL^{2-}$	2	14	$11.82 \pm 0.07$	-
$(CH_3)_4N^+ + 2 H^+ + L^{4-} = (CH_3)_4NH_2L^-$	3	18	$16.45 \pm 0.07$	-
$(CH_3)_4N^+ + 3 H^+ + L^{4-} = (CH_3)_4NH_3L^{0(aq)}$	4	20	$18.59 \pm 0.24$	-
Parameter			$Y_\theta$	$\frac{\partial Y}{\partial T}$
$c_0$			0.165 <sup>d</sup>	
$c_1$			$0.200 \pm 0.008$ <sup>c</sup>	$-0.0011 \pm 0.0003$ <sup>c</sup>

<sup>a</sup>  $p^*$  and  $z^*$  are parameters of Equation (18); <sup>b</sup> Taken from Table 3 and kept constant during calculations; <sup>c</sup> s (standard deviation); <sup>d</sup> kept fixed during calculations; standard uncertainties:  $u(T) = 0.1$  K,  $u(p) = 1$  kPa.

Data reported in Table 5 indicate that the ligand anion ( $L^{4-}$ ) interaction with the sodium cation is stronger than that with the potassium and tetramethylammonium cations. In fact, the formation constant values of the ML species at infinite dilution are  $\log K = 2.50$ , 2.20 and 1.89, respectively. Moreover, for the sodium cation only, the presence of a dinuclear  $M_2L$  species was evidenced. The stepwise formation constant value of this species can be obtained by subtracting the value of the NaL from that of the Na<sub>2</sub>L, thus, is  $\log K = 3.01 - 2.50 = 0.51$ . Similarly, the stepwise formation constant values of the protonated

species can be obtained by subtracting the values of the protonation constants from those of the  $MHL$  species. Values so obtained decrease linearly with the increasing number of the protons of the species, the slopes obtained for the three cations are not statistically different from each other. Additionally, in this case, similar trends have been observed in the literature [52,55].

### 3.2. Interaction of L-glutamic-N,N-diacetic Acid with $Cd^{2+}$

Interaction of L-glutamic-N,N-diacetic acid with  $Cd^{2+}$  was studied by means of potentiometric titrations at different ionic strength values and at  $T = 298.15$  K in aqueous NaCl solutions. The data analysis was performed using the BSTAC4 computer program to analyze titrations carried out at different ionic strength values to obtain the formation constants of the  $Cd_iHL$  species at infinite dilution together with the relative ionic strength dependence parameters of Equation (9), thus, no experimental data at different ionic strength values are available. During this process, the protonation constants of  $Glda^{4-}$  relative to the NaCl medium were kept fixed to the values reported in Table 3; the equilibrium constants of the  $Cd^{2+}/Cl^-$  and  $Cd^{2+}/OH^-$  species were taken from [56]. The results of the data analysis performed in the pH range  $2 \leq pH \leq 11$  are summarized in Table 6, reporting the formation constant at infinite dilution ( $\log \beta_{ij}^0$ ) of the found species, the ionic strength dependence parameters ( $C_{ij}$ ), the maximum of formation of each species (Max (%)) and the pH value at which it was achieved.

The choice of the best speciation model is always a challenging task. In this work, a statistical approach based on the variance ratio and goodness-of-fit (GOF) criteria was used for this purpose. According to Crea et al. [57], the criteria used to select the chemical model are: (a) the value of the variance ratio among different speciation models; (b) the simplicity of the model (other minor species can be added to the chosen ones but the model becomes unrealistically complicated); (c) the likelihood of the proposed species, in particular, in relation to similar systems.

The mean deviation of the whole fit (i.e., the average deviation of the experimental e.m.f. of each datum point, in absolute value, from the fitted value) is 3.7 mV. This value is acceptable for a complex system, also considering the variable ionic strength and the wide pH range considered.

**Table 6.** Overall metal ligand complex formation constants, ionic strength dependence parameters (both in the molar,  $C_{ij}$ , and in the molal concentration scale,  $\Delta\varepsilon_{ij}$ ) and details on the formation percentages ( $C_{Cd} = 0.4$  mmol  $dm^{-3}$ ;  $C_L = 0.97$  mmol  $dm^{-3}$ ,  $I = 0.5$  mol  $dm^{-3}$ ) of  $Cd^{2+}/GLDA^{4-}$  species for the selected speciation model obtained in the pH range  $2.0 < pH < 10.0$  at  $T = 298.15$  K and  $p = 0.1$  MPa.

Equilibrium	$z^*$ <sup>a</sup>	$\log \beta_{ij}^0$	$C_{ij}$	$\Delta\varepsilon_{ij}$	Max (%)	pH
$Cd^{2+} + L^{4-} = CdL^{2-}$	16	$12.68 \pm 0.03$ <sup>b</sup>	$-0.32 \pm 0.06$ <sup>b</sup>	$-0.31$ <sup>c</sup>	98	8.6
$Cd^{2+} + H^+ + L^{4-} = CdHL^-$	20	$17.61 \pm 0.03$	$0.66 \pm 0.06$	0.65	50	4.2
$Cd^{2+} + 2 H^+ + L^{4-} = CdH_2L^{0(aq)}$	22	$20.76 \pm 0.05$	$1.81 \pm 0.10$	1.77	27	2.9
$2 Cd^{2+} + L^{4-} = Cd_2L^{0(aq)}$	24	$17.52 \pm 0.05$	$1.49 \pm 0.07$	1.46	5	4.7
$Cd^{2+} + H_2O + L^{4-} = Cd(OH)L^{3-} + H^+$	10	$1.77 \pm 0.04$	$-2.10 \pm 0.14$	$-2.05$	18	10.0

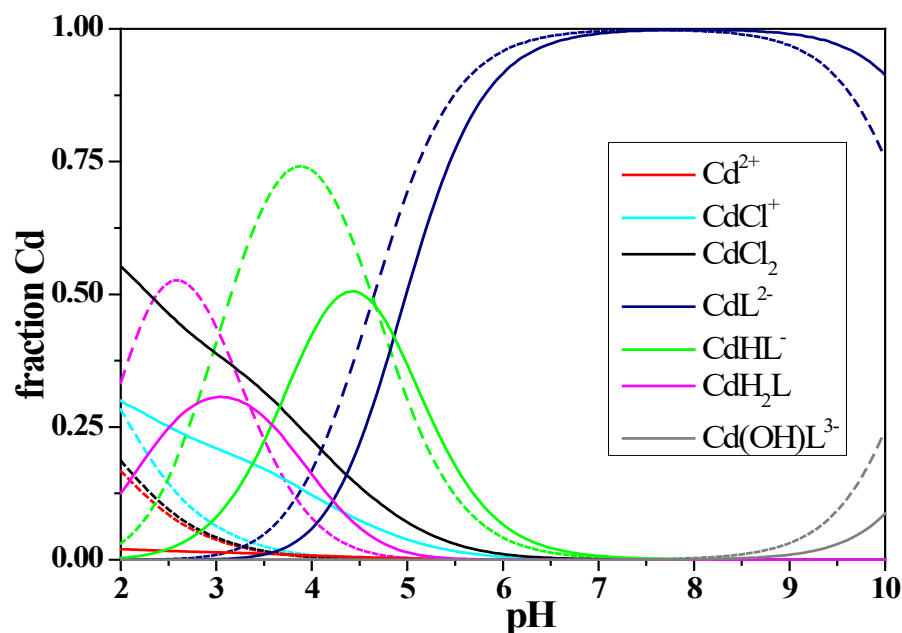
<sup>a</sup>  $p^*$  and  $z^*$  are parameters of Equation (18); <sup>b</sup> s (standard deviation); <sup>c</sup> error to be associated is the same as the corresponding  $C_{ij}$  value; standard uncertainties:  $u(T) = 0.1$  K,  $u(p) = 1$  kPa,  $u(\text{Max}) = 1$  %;  $u(\text{pH}) = 0.1$ .

The speciation model reported in Table 6 seems to be the most likely for the system under analysis. Together with these species reported, the possible presence of the  $M_3L$  species was also tested ( $\log \beta_{03}^0 = 21.92 \pm 0.05$  and  $C_{03} = 2.33 \pm 0.11$ ) but the formation percentage reached was significant (~15%) only in a few titrations with a high  $C_{Cd}:C_{GLDA}$  ratio to include it in the final speciation scheme. Moreover, limiting the pH to the acidic range ( $2.0 < pH < 5.0$ ), several minor species were determined. Some examples of possible

speciation schemes refined in the mentioned pH range are reported as Supplementary Materials (Table S2) and also contain polynuclear species such as  $M_2H_3L$ ,  $M_2H_2L_2$ ,  $M_2H_3L_3$ ,  $M_3H_2L_2$ ,  $M_3H_3L_3$ ,  $M_3H_4L_3$ ,  $M_3H_2L_4$  and  $M_4H_2L_4$ . Interestingly, the value of the stability constant of the ML species obtained in such trials is always considerably lower than that refined in the selected speciation scheme because the value was refined in a pH range where the formation of this species never reaches the maximum, thus, is underestimated.

Although their presence in the final speciation scheme was not taken into account, the formation of the  $M_3L$  and other minor polynuclear species mentioned cannot be excluded. However, considering the stoichiometry of the species, their presence is not fundamental for the assessment of speciation in natural fluids because their formation may occur only in experimental conditions hardly found in nature, namely  $C_{Cd} > C_{GLDA}$  and total concentrations exceeding  $2.0 \text{ mmol dm}^{-3}$ . In any case, the identification of polynuclear species is always an interesting task, but dedicated experiments with proper techniques (e.g., ESI-MS) should be performed to confirm or exclude the presence of such species.

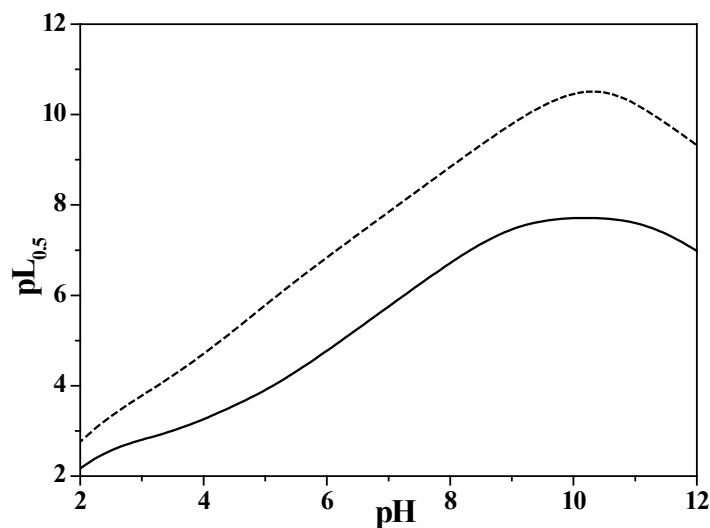
The importance of the complex formation constants of the  $Cd^{2+}/Glda^{4-}$  species is depicted in Figure 2, where the speciation diagram of the system is given at low ionic strength ( $I = 0.05 \text{ mol kg}^{-1}$ , dashed line) and at the ionic strength of seawater ( $I = 0.72 \text{ mol kg}^{-1}$ , solid line). At low ionic strength values, the importance of the chloro complexes is marginal so that the complexation with the ligand is more relevant. Moreover, as ionic strength increases the complexation is shifted to more alkaline pH values by  $\sim 0.5 \text{ pH}$  units. In the experimental conditions selected ( $C_{Cd} = 10^{-5} \text{ mol kg}^{-1}$ ;  $C_L = 10^{-3} \text{ mol kg}^{-1}$ ), the  $CdH_2L^0_{(aq)}$  species reach the maximum formation percentages at  $\text{pH} = 2.63$  (52 %) and  $\text{pH} = 3.07$  (31 %) at  $I = 0.05$  and  $0.72 \text{ mol kg}^{-1}$ , respectively, whereas for  $CdHL^-$  it is at  $\text{pH} = 3.92$  (74 %) and  $\text{pH} = 4.43$  (50 %) in the same conditions. This also applies to the  $CdL^{2-}$  species, but the formation percentage reaches 100 % in both cases, and to the  $Cd(OH)L^{3-}$  species, even if the maximum of formation is not reached in the selected pH range. The  $Cd_2L^0_{(aq)}$  species is not formed in these experimental conditions.



**Figure 2.** Speciation diagram of the  $Cd^{2+}/Glda^{4-}$  system is given at low ionic strength ( $I = 0.05 \text{ mol kg}^{-1}$ , dashed line) and at ionic strength of seawater ( $I = 0.72 \text{ mol kg}^{-1}$ , solid line) at  $T = 298.15 \text{ K}$  and  $p = 0.1 \text{ MPa}$ . Experimental conditions:  $C_{Cd} = 10^{-5} \text{ mol kg}^{-1}$ ;  $C_L = 10^{-3} \text{ mol kg}^{-1}$ . Species:  $Cd^{2+}$  (red),  $CdCl^+$  (cyan),  $CdCl_2^0_{(aq)}$  (black),  $CdL^{2-}$  (navy),  $CdHL^-$  (green),  $CdH_2L^0_{(aq)}$  (magenta),  $Cd(OH)L^{3-}$  (gray).

Apart from the numerical value of the equilibrium constants, an operational measure to quantify the sequestering ability and, therefore, select the most suitable chelating agent for the sequestration of a given metal cation is required. Many parameters have been proposed and most of them are reviewed by Bazzicalupi et al. [58] and Gama et al. [59]. In this work, the sequestering ability of GLDA toward  $\text{Cd}^{2+}$  is assessed by computing  $\text{pL}_{0.5}$ . The value of the  $\text{pL}_{0.5}$  represents the antilogarithm of the total ligand concentration required to sequester 50 % of the metal cation present as the trace level in each condition. Crea et al. [39] pointed out the similarity of  $\text{pL}_{0.5}$  and the Schwarzenbach's ( $K'$  or apparent,  $K_{\text{app}}$ ) constant; in this light, it is noticeable that the shape of the function reported in Figure 3 is very similar to that reported in the technical report by AkzoNobel, computed starting from NIST data [60], for the apparent formation constants (i.e., Schwarzenbach) of other cations with weak hydrolysis, such as  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ . However, the use of  $\text{pL}_{0.5}$  has some relevant advantages over the apparent constants, especially in very complex systems. Among them, it is significant that it is easier also to be determined by non-specialist users by means of dedicated computer programs, without any deep knowledge of the treatment of simultaneous equilibria and mass balance equations.

The dependence of  $\text{pL}_{0.5}$  of GLDA toward  $\text{Cd}^{2+}$  on pH at  $I = 0.05$  (dotted line) and  $0.72$  (solid line)  $\text{mol kg}^{-1}$  is displayed in Figure 3. It can be noted that  $\text{pL}_{0.5}$  increases almost linearly with pH at both ionic strength values, inverting the trend at  $\text{pH} \sim 10$  due to the hydrolysis of the  $\text{Cd}^{2+}$  cation. Tracing what was found for the formation percentage of complex species noted in Figure 2, complexation is generally greater at low ionic strength values; in terms of  $\text{pL}_{0.5}$  it may be noted that values at  $I = 0.05 \text{ mol kg}^{-1}$  are higher than those at  $I = 0.72 \text{ mol kg}^{-1}$  by one or two orders of magnitude. In this context, it should be noted that experimental data were analyzed up to  $\text{pH} = 10$ ; no information about further complex species with stoichiometry  $\text{M}_j(\text{OH})_2\text{L}$ , whose formation occurs at  $\text{pH} > 10$ , are available, but their presence seems very unlikely in the case of  $\text{Cd}^{2+}$ .



**Figure 3.** Dependence of the  $\text{pL}_{0.5}$  in pH at  $I = 0.05 \text{ mol kg}^{-1}$  (dotted line) and  $I = 0.72$  (solid line)  $\text{mol kg}^{-1}$  at  $T = 298.15 \text{ K}$  and  $p = 0.1 \text{ MPa}$ .

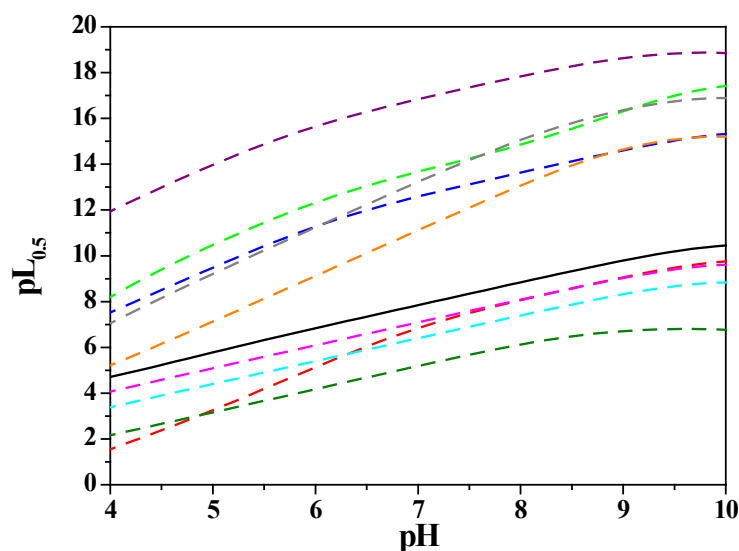
### 3.3. Comparisons with the Literature Data

Minor discrepancies reported for protonation constants have already been discussed, and data are sufficient to assess a robust dependence on temperature and ionic strength.

Regarding the  $\text{Cd}^{2+}/\text{Glda}^{4-}$  complex formation constants, direct formation constant comparisons with the ones already published can hardly be made since so few values were reported in the literature, and typically only in a single experimental condition. For example, Begun et al. [7] indicated the formation of four complex species, namely  $\text{CdL}^{2-}$ ,  $\text{CdHL}^-$ ,  $\text{CdH}_2\text{L}^{0\text{aq}}$  and  $\text{Cd}(\text{OH})\text{L}^{3-}$ , in  $\text{KCl}_{\text{aq}}$  at  $I = 0.1 \text{ mol dm}^{-3}$  and  $T = 298.15 \text{ K}$ , but all the

data were obtained by working in only one experimental condition of equimolar concentration of metal cation and GLDA ( $1 \text{ mmol dm}^{-3}$ ). Although the four cited species were also found in the current work, the formation constant values suffer from some numerical discrepancies likely deriving from the different ionic mediums and, thus, from the different values of the protonation constants used. Moreover, from the distribution diagram reported by Begun et al. [7], it seems that the  $\text{Cd}^{2+}/\text{Cl}^-$  species were not considered in the speciation model so that the formation constant values for the  $\text{Cd}^{2+}/\text{Glda}^{4-}$  species must be considered as conditional. Besides the mentioned differences in the evaluation of equilibrium constants, distribution diagrams reported in Figure 2 are comparable to the ones found by Begun et al. [7] but only if, in our case, the formation percentages of the  $\text{Cd}^{2+}$ ,  $\text{CdCl}^+$  and  $\text{CdCl}_2^0(\text{aq})$  are summed up.

By taking from the literature the most reliable data for the interaction of  $\text{Cd}^{2+}$  with some complexones, such as ethylenediaminetetraacetic acid (EDTA [61]), triethylenetetraaminehexaacetic acid (TTHA, [62]), ethylenediamine- $N,N'$ -disuccinic acid ( $S,S$ -EDDS, [63]),  $N$ -(2-hydroxyethyl)iminodiacetic acid (HIDA, [64]), nitrilotriacetic acid (NTA, [65]), Cyclohexanediaminetetraacetic acid (CDTA, [60]), diethylenetriaminepentaacetic acid (DTPA, [66]), ethylene glycol-bis( $\beta$ -aminoethyl ether)- $N,N,N',N'$ -tetraacetic acid (EGTA, [67]) and methylglycindiacycetic acid (MGDA, [68]) the  $\text{pL}_{0.5}$  was computed in the same conditions of temperature ( $T = 298.15 \text{ K}$ ) and ionic strength ( $I = 0.1 \text{ mol dm}^{-3}$ ) in the pH window of natural waters ( $4 \leq \text{pH} \leq 10$ , Figure 4). The plots obtained are almost parallel, depending on the protonation constants of the complexone, with a maximum between  $\text{pH} \sim 9$  and  $\text{pH} \sim 10$ . Complexones with more binding sites display higher  $\text{pL}_{0.5}$  values, as in the case of TTHA (ten binding sites) and DTPA (eight binding sites). Quite surprisingly, CDTA (with six binding sites) shows the highest  $\text{pL}_{0.5}$  values among all complexones. GLDA, with five binding sites and depicted with a solid line, has an average value between MGDA (four binding sites) and EGTA (six binding sites). Interestingly, among the new generation biodegradable chelants, namely MGDA, NTA, EDDS and HIDA, GLDA achieves the highest  $\text{pL}_{0.5}$  value.



**Figure 4.** Comparison of the  $\text{pL}_{0.5}$  of various complexones toward  $\text{Cd}^{2+}$  at  $I = 0.1 \text{ mol dm}^{-3}$  and  $T = 298.15 \text{ K}$  at different pH values. Complexones: EDDS (red), TTHA (green), EDTA (blue), DTPA (gray), MGDA (magenta), NTA (cyan), EGTA (orange), CDTA (purple), HIDA (olive), GLDA (black).

#### 4. Conclusions

Although the industrial employment of GLDA as a sustainable and biodegradable new generation chelating agent seems promising as an alternative to the traditional chelants, the scientific literature on this topic is still not in agreement. With the aim of

providing a solid foundation for the development of sustainable strategies for water and soil remediation, here we report a systematic study on GLDA protonation thermodynamics and interaction with  $\text{Cd}^{2+}$  in an aqueous solution. New data in a standard state (i.e., infinite dilution) are given here, together with parameters useful for calculating protonation thermodynamics, the formation constants and the sequestering ability of GLDA toward  $\text{Cd}^{2+}$  at the pH and ionic strength values of most natural fluids. From the data discussed here, it can be claimed that a deprotonated ligand is involved in four successive protonation steps, the first being related to the nitrogen atom and the following to the carboxylate groups. In addition, the medium effect was interpreted by means of the so-called “pure water model”; according to this approach, the dependence on ionic strength in different media depends on the formation of weak species between the ligand, or its partially protonated forms, with the cations of the supporting electrolyte. The interaction of the  $\text{Glda}^{4-}$  with  $\text{Na}^+$  results were greater with respect to the ones displayed by  $\text{K}^+$  and  $(\text{CH}_3)_4\text{N}^+$ , and a set of thirteen weak complexes, with temperature and ionic strength coefficients, was obtained.

The whole of the data collected on the  $\text{Cd}^{2+}/\text{Glda}^{4-}$  system made it possible to determine a speciation scheme featured by five complex species, namely  $\text{CdL}^{2-}$ ,  $\text{CdHL}^-$ ,  $\text{CdH}_2\text{L}^0_{(\text{aq})}$ ,  $\text{Cd}(\text{OH})\text{L}^{3-}$  and  $\text{Cd}_2\text{L}^0_{(\text{aq})}$ . Furthermore, stability constants at different ionic strength values and at  $T = 298.15$  K were assessed. As discussed, the speciation of  $\text{Cd}^{2+}/\text{Glda}^{4-}$  is largely influenced by ionic strength, as it is the  $\text{Cd}^{2+}/\text{Cl}^-$  species relevant only in specific conditions (i.e., in acidic solution and at high chloride concentration values). The  $\text{pL}_{0.5}$  calculations also made it possible to ascertain that the sequestering ability of GLDA toward cadmium cation increases with the pH up to a value of pH ~10 highlighting, on the other hand, that the presence of a background electrolyte, especially chloride, exerts a negative effect on sequestration.

GLDA, among the new generation biodegradable chelants, featuring the highest  $\text{pL}_{0.5}$  value, is a good alternative to traditional chelants for sustainable  $\text{Cd}^{2+}$  removal from waters. Therefore, the implementation of GLDA in strategies and/or materials specifically designed for this purpose deserves further investigation.

**Supplementary Materials:** Table S1. Experimental values of the protonation constants of L-glutamic-N,N-diacetic acid (L-GLDA) in different ionic media, at different temperatures and at different ionic strengths. Table S2. Overall metal ligand complex formation constants, ionic strength dependence parameters and fit statistics of  $\text{Cd}^{2+}/\text{Glda}^{4-}$  system for some proposed speciation models obtained in the pH range  $2.0 < \text{pH} < 5.0$ ; excel spreadsheet: Data couples ( $\text{cm}^3$  of titrant vs. e.m.f.) obtained for all the potentiometric titrations analyzed in this work;

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