Cation, Anion and Ion-Pair Complexes with a G-3 Poly(ethylene imine) Dendrimer in Aqueous Solution

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Abstract: The G-3 poly(ethylene imine) ligand L2 shows a multifaceted coordination ability, being able to bind metal cations, anions and ion-pairs. The equilibrium constants for the formation of metal (Cu$^{2+}$, Zn$^{2+}$), anion (SO$_4^{2-}$) and ion-pair (Cu$^{2+}$/SO$_4^{2-}$) complexes were determined in 0.1 M Me$_4$NCl aqueous solution at 298.1 ± 0.1 K by means of potentiometric titrations. Thanks to its dendrimeric nature, L2 can form highly nucleated metal complexes, such as Cu$_5$L$_2$$^{10+}$ and Zn$_4$L$_2$$^{8+}$, in successive and well-defined complexation steps. Protonated forms of L2 give rise to relatively weak anion complexes with SO$_4^{2-}$, but the addition of Cu$^{2+}$ significantly enhances the binding ability of the ligand toward this anion below pH 9. In more alkaline solutions, an opposite trend is observed. The coordination properties of L2 are discussed with the support of modelling calculations. According to results, L2 is a promising molecule for the preparation of solid supported materials for the recovery of cations and anions from aqueous media and/or for applications in heterogeneous catalysis.

Keywords: copper; zinc; dendrimers; poly(ethylene imine); polynuclear complexes; anion complexes; ion-pair complexes

1. Introduction

In recent papers, we showed that the G-2 poly(ethylene imine) dendrimer L1 (Figure 1) and its variously protonated forms are able to assemble stable cation, anion and ion-pair complexes in aqueous solution [1–4]. Such ability appears to be a propagation and an enhancement of the properties of the parent ligand tris(2-aminoethyl)amine (tren), which is historically known to bind metal complexes and, more recently, has also been accredited as a rather efficient anion receptor [5]. Indeed, regarding the coordination of metal ions, while tren forms stable mononuclear complexes, L1 can bind two metal ions such as Ni$^{2+}$, Zn$^{2+}$ and Cd$^{2+}$ and up to three Cu$^{2+}$ and Hg$^{2+}$ ions [1,4]. L1 and its metal complexes are also able to bind inorganic anions [2] as well as the anionic forms of AMP, ADP and ATP nucleotides acting as catalysts that enhance significantly ATP dephosphorylation in aqueous solution [3]. Construction of a third generation of ethylamino branches around L1 gave rise to the G-3 poly(ethylene imine) dendrimer L2 (Figure 1) that is also able to bind anions, such as PO$_4^{3-}$, P$_2$O$_7^{4-}$ and P$_3$O$_{10}^{5-}$, and AMP, ADP and ATP nucleotides. In particular, L2 showed an unprecedented behaviour toward ATP, the dendrimer being able to enhance or inhibit dephosphorylation of the nucleotide depending on the solution pH [6].
Despite the discovery of such properties towards nucleotide and phosphate type anions, the ability of L2 to bind metal cations, inorganic anions and ion-pairs remains unexplored. Actually, L2 is a very interesting ligand for the formation of metal complexes, in particular of polynuclear assemblies, since it contains a large number (22) of nitrogen donor atoms in its molecular structure, and accordingly, it should be able to form complexes of greater nuclearity than the smaller homologous L1. It is worth of note that there is a great deal of interest toward polynuclear metal complexes, especially for their catalytic properties and/or for their use in the generation of metal nanoparticle-based catalysts [7–10].

This has aroused our interest in performing a detailed analysis of the complexation equilibria involving L2, metal cations and anions in aqueous solution. As previously noted [1], to study similar complexation systems is an arduous task, due to the many equilibria involving the stepwise coordination of several metal ions involving several protonation states for each complexation step. Attempts to perform the speciation of complex systems and determining the equilibrium constants for complexation equilibria with other poly(ethylene imine) dendrimers were made by considering the repeating triamine units of the dendrimer as identical independent ligand molecules, under the implicit assumption that these repeating units were uniformly distributed in solution, in contrast to their actual localization within the same dendrimer molecule [11]. These studies were performed under conditions approaching the ligand coordinative saturation, the metal-to-triamine unit ratios being closed to 1:1 and extending it, at most, up to 1:4.

Despite such an approximation, the results of these studies can be functional for the purposes for which they are developed, although they furnish an incomplete picture of the complexation systems. In particular, this approach leads to the identification of a limited number of complex species relative to the many that these dendrimers can form. For instance, dendrimers containing large numbers of amino groups are expected to bind metal ions even when they are extensively protonated. Some of the missing species could have interesting properties, like the ability of highly protonated Zn(II) complexes with L1 to promote the binding and the dephosphorylation of ATP [3].

In this paper, we report the results of a detailed analysis of the complexation systems formed by L2 with Zn$^{2+}$ and Cu$^{2+}$ that led to the identification of 35 and 42 complex species for Zn$^{2+}$ and Cu$^{2+}$, respectively, the ligand achieving the stepwise coordination of 4 Zn$^{2+}$ or 5 Cu$^{2+}$ ions. Once these complexation systems were clearly defined, we analysed the ability of L2 to interact with SO$_4^{2−}$ both in the absence and in the presence of Cu$^{2+}$.

**2. Results and Discussion**

2.1. Formation of Metal Complexes

Speciation of L2/Cu$^{2+}$ and L2/Zn$^{2+}$ complex systems and determination of the relevant stability constants were performed by means of pH-metric (potentiometric) titrations (0.1 M Me$_4$NCl, 298.1 ± 0.1 K) and analysis of the associated data by means of the computer program HYPERQUAD [12].
298.1 ± 0.1 K) and analysis of the associated data by means of the computer program HYPERQUAD [12] which furnished the stability constants collected in Tables 1 and 2 for Cu²⁺ and Zn²⁺, respectively. Distribution diagrams of the complexes formed are reported in Figures S1 and S2.

As shown by these tables, the G-3 dendrimer L2 is able to bind in successive steps from one to five Cu²⁺ cations and from one to four Zn²⁺ ions. According to the presence of many (22) nitrogen donor atoms in the ligand, all complexes but Cu₅L₂¹⁰⁺ are able to bind protons, and the number of protonated species they form decreases with increasing complex nuclearity. It was previously reported that protonation of the primary amine groups of L2 is associated with protonation constants logK ≥ 8.3 [6]. Considering this value as the limiting value for protonation of primary amine groups also in L2 complexes, we can deduce from the equilibrium data in Table 1 that, in CuL₂²⁺, there are nine primary amine groups, out of 12, that are not involved in metal coordination. By similar reasoning, and taking into account the experimental errors on the determined equilibrium constants, the number of uncoordinated primary nitrogens can be reasonably estimated as six in Cu₂L₂⁴⁺, five in Cu₃L₂⁶⁺, three in Cu₄L₂⁸⁺, and none in Cu₅L₂¹⁰⁺. The equilibrium constants for the successive binding of the first and the second Cu²⁺ ions are very high (logK = 23.66 and 22.9, Table 1) and consistent with the stability of hexacoordinated Cu²⁺ complexes of polyamines [13,14]. Accordingly, the first two Cu²⁺ ions binding L2 should be coordinated by three primary and three secondary amine groups near the surface of the G-3 dendrimer. The third coordination stage causes a greater involvement of the inner dendrimer region, since only one primary amine group is involved in the equilibrium Cu₅L₂¹⁰⁺ + Cu²⁺ = Cu₃L₂⁸⁺. This appears to be a poorly favourable coordination step as shown by the surprisingly low value of the corresponding equilibrium constant (logK = 10.0, Table 1).

Table 1. Stability constants of Cu²⁺ complexes with L2. 0.1 M Me₄NCl, 298.1 ± 0.1 K. Values in parentheses are standard deviation on the last significant figure.

| Equilibria | logK  |
|------------|-------|
| Cu²⁺ + L₂ = CuL₂²⁺ | 23.66 (5) |
| CuL₂²⁺ + 2H⁺ = CuH₂L₂²⁺ | 22.88 (7) |
| CuH₂L₂²⁺ + H⁺ = CuH₃L₂²⁺ | 9.93 (5) |
| CuH₃L₂²⁺ + H⁺ = CuH₄L₂²⁺ | 10.07 (5) |
| CuH₄L₂²⁺ + H⁺ = CuH₅L₂²⁺ | 9.42 (3) |
| CuH₅L₂²⁺ + H⁺ = CuH₆L₂²⁺ | 9.21 (7) |
| CuH₆L₂²⁺ + H⁺ = CuH₇L₂⁹⁺ | 9.09 (7) |
| CuH₇L₂⁹⁺ + H⁺ = CuH₈L₂¹₀⁺ | 8.63 (5) |
| CuH₈L₂¹₀⁺ + H⁺ = CuH₉L₂¹¹⁺ | 8.50 (4) |
| CuH₉L₂¹¹⁺ + H⁺ = CuH₁₀L₂¹²⁺ | 8.13 (4) |
| CuH₁₀L₂¹²⁺ + H⁺ = CuH₁₁L₂¹³⁺ | 7.49 (4) |
| CuH₁₁L₂¹³⁺ + H⁺ = CuH₁₂L₂¹⁴⁺ | 5.87 (4) |
| CuH₁₂L₂¹⁴⁺ + H⁺ = CuH₁₃L₂¹⁵⁺ | 5.18 (4) |
| CuH₁₃L₂¹⁵⁺ + H⁺ = CuH₁₄L₂¹⁶⁺ | 3.94 (5) |
| CuH₁₄L₂¹⁶⁺ + H⁺ = CuH₁₅L₂¹⁷⁺ | 2.59 (5) |
| CuH₁₅L₂¹⁷⁺ + H⁺ = CuH₁₆L₂¹₈⁺ | 2.89 (6) |
| 2Cu²⁺ + L₂ = Cu₂L₂²⁺ | 46.53 (7) |
| Cu₂L₂²⁺ + Cu²⁺ = Cu₃L₂⁴⁺ | 22.9 (1) |
| Cu₃L₂⁴⁺ + H⁺ = Cu₄H₂L₂⁴⁺ | 11.51 (6) |
| Cu₄H₂L₂⁴⁺ + H⁺ = Cu₅H₃L₂⁶⁺ | 10.20 (7) |
| Cu₅H₃L₂⁶⁺ + H⁺ = Cu₆H₄L₂⁸⁺ | 9.24 (7) |
| Cu₆H₄L₂⁸⁺ + H⁺ = Cu₇H₅L₂⁸⁺ | 9.61 (6) |
| Cu₇H₅L₂⁸⁺ + H⁺ = Cu₈H₆L₂⁹⁺ | 8.31 (7) |
| Cu₈H₆L₂⁹⁺ + H⁺ = Cu₉H₇L₂¹₀⁺ | 8.22 (7) |
| Cu₉H₇L₂¹₀⁺ + H⁺ = Cu₁₀H₈L₂¹₁⁺ | 8.18 (7) |

Such a drop of the metal ion binding constant is, most likely, determined by an important structural rearrangement that the very stable Cu₅L₂¹⁰⁺ complex must bear to accommodate the third Cu²⁺ ion. Conversely, Cu₃L₂⁶⁺ displays a greater binding ability toward Cu²⁺ than Cu₂L₂⁴⁺ (Cu₃L₂⁶⁺ + Cu²⁺ = Cu₄L₂⁸⁺, logK = 16.0, Table 1), that is, the coordination of the third Cu²⁺ ion is not very favourable but generates the structural conditions for a favourable continuation of the
stepwise binding process. Two of the 5 free primary amine groups of Cu3L26+ become coordinated in Cu4L28+; while no primary amine group appears to be available for protonation in Cu3L210+. The formation of the latter from the tetranuclear complex is accompanied by a small equilibrium constant (Cu4L28+ + Cu2+ = Cu3L210+, logK = 9.4, Table 1) in agreement with the high electrostatic repulsion exerting between the five metal ions and the reduced number of donor atoms remaining available for coordination in Cu4L28+. As a matter of fact, the ligand is not able to fulful the coordination sphere of all five metal ions in Cu5L210+ and facile dissociation of coordinated water molecules generates the hydroxo complex [Cu3L2(OH)2]8+.

In contrast to Cu2+, in the case of Zn2+ complexation, the equilibrium constants for the successive binding of metal ions to form ZnL22+, Zn2L24+, Zn3L26+ and Zn4L28+ (logK = 17.8, 13.2, 11.0, 10.7, Table 2) display a more regular trend. The loss of stability from the mono- to the binuclear complex (logK = 17.8, 13.2, Table 2) is greater than for the corresponding equilibria with Cu2+. Nevertheless, also the stability constants for the formation of ZnL22+ and Zn2L24+ are consistent with the stability of hexacoordinated Zn2+ complexes with polyamines [15,16]. According to the criterium based on complex protonation constants, the number of uncoordinated primary amine group should be 9 in ZnL22+ and 6 in Zn2L24+, in agreement with a coordination sphere constituted by three primary and three tertiary nitrogen atoms for both metal ions. Binding of the third Zn2+ ion takes place with further decrease of stability (Zn2L24+ + Zn2+ = Zn3L26+, logK = 11.0, Table 2). At this stage, another three primary amine groups become involved in metal binding, suggesting a similar hexacoordination for all three metal ions in Zn3L26+. An insignificant decrease of binding constant is instead observed at the fourth coordination step (Zn3L26+ + Zn2+ = Zn4L28+, logK = 10.7, Table 2) even though an important reorganization of the trinuclear complex must occur to accommodate the fourth Zn2+ ion. According to protonation data in Table 2, two primary nitrogen atoms should remain uncoordinated in Zn4L28+.

Table 2. Stability constants of Zn2+ complexes with L2. 0.10 M Me4NCl, 298.1 ± 0.1 K. Values in parentheses are standard deviation on the last significant figure.

| Equilibria | logK  | Equilibria | logK  |
|------------|------|------------|------|
| Zn2L22+ + L2 = ZnL24+ | 17.18 (5) | Zn2H4L210+ + H+ = Zn2H3L211+ | 8.13 (8) |
| ZnL22+ + 2H+ = ZnH3L24+ | 22.50 (8) | Zn2H3L211+ + H+ = Zn2H3L212+ | 7.36 (7) |
| ZnH3L24+ + H+ = ZnH4L25+ | 10.04 (5) | ZnH3L212+ + H+ = ZnH3L213+ | 6.47 (5) |
| ZnH3L25+ + H+ = ZnH4L26+ | 9.59 (6) | Zn2L24+ + Zn2+ = Zn3L26+ | 41.36 (5) |
| ZnH3L26+ + H+ = ZnH4L27+ | 10.01 (7) | Zn2H3L24+ + Zn2+ = Zn3L26+ | 11.0 (1) |
| ZnH4L27+ + 2H+ = ZnH5L29+ | 18.14 (7) | ZnH3L24+ + 2H+ = ZnH5L29+ | 22.52 (6) |
| ZnH5L29+ + H+ = ZnH6L31+ | 8.25 (6) | ZnH3L24+ + H+ = ZnH5L29+ | 9.34 (8) |
| ZnH5L31+ + H+ = ZnH6L32+ | 8.64 (7) | ZnH4L26+ + H+ = ZnH6L32+ | 8.12 (8) |
| ZnH6L32+ + H+ = ZnH7L34+ | 7.97 (6) | ZnH4L26+ + H+ = ZnH6L32+ | 8.00 (8) |
| ZnH7L34+ + H+ = ZnH8L36+ | 6.92 (5) | ZnH4L26+ + H+ = ZnH6L32+ | 6.94 (6) |
| ZnH8L36+ + H+ = ZnH9L38+ | 5.75 (4) | ZnH5L28+ + H+ = ZnH6L32+ | 6.26 (6) |
| ZnH9L38+ + H+ = ZnH10L40+ | 5.38 (5) |

To get insight into the structural properties of these Zn2+ polynuclear complexes, we performed molecular modelling calculations on Zn2L24+, Zn3L26+ and Zn4L28+ in a simulated implicit water environment. The lower energy structures obtained for these complexes are shown in Figure 2. According to these structures, in Zn2L24+ (Figure 2a) and Zn3L26+ (Figure 2b) each metal ion is coordinated, in a distorted octahedral environment, to six nitrogen atoms pertaining to one arm of the ligand originating from the central tertiary amine group. In agreement with the deductions drawn above from the equilibrium constants, the number of primary nitrogen atoms remaining not
coordinated is six in $\text{Zn}_2\text{L}_2^{4+}$ (Figure 2a) and three in $\text{Zn}_3\text{L}_2^{6+}$ (Figure 2b). Indeed, the addition of the fourth $\text{Zn}^{2+}$ ion causes a major rearrangement of the trinuclear complex. The ligand displays a great ability to minimize the electrostatic repulsion between metal cations bringing them at long distance from each other (Figure 2c). Only one of the $\text{Zn}^{2+}$ ions retains the octahedral coordination environment seen in the trinuclear complex, while the other three metal cations are: one pentacoordinated by ligand nitrogen atoms, one pentacoordinated by four ligand donors and a water molecule, one tetracoordinated by two ligand donors and two water molecules. The last coordination environment requires some cautionary considerations. In this complex unit, the ligand forms an 8-membered chelate ring including a not coordinated nitrogen atom. A similar arrangement is unlikely to occur in a real solution, since chelate rings of such size are poorly stable. In the simulated implicit water environment of our calculations, however, an overestimation of electrostatic repulsions could have forced the $\text{Zn}^{2+}$ ion to stay as far as possible from the other three cations, instead of involving the third nitrogen atom in the formation of two stable 5-membered chelate rings, which is the situation that we expect to occur in water. Nevertheless, the calculated structure of $\text{Zn}_4\text{L}_2^{8+}$ (Figure 2c) seems very representative of the overall organization of this complex, as shown by the fact that it implicates the presence of two not coordinated primary nitrogen atoms in agreement with the results deduced above from equilibrium data.

2.2. Formation of Anion and Ion-Pair Complexes

The detailed analysis of metal complexation equilibria with L2 makes it possible to further investigate such equilibrium systems. For instance, it is possible to analyse the ability of L2 complexes to interact with other species in the environment. We have already seen that protonated forms of L2 can bind $\text{PO}_4^{3−}$, $\text{P}_2\text{O}_7^{4−}$, $\text{P}_3\text{O}_{10}^{5−}$, and nucleotides (AMP, ADP, ATP) anions in solution [6], and we have already seen that the G-2 dendrimer L1 is able to form both anion and ion-pair complexes [2,3]. We have now studied the equilibria involving L2 and $\text{SO}_4^{2−}$ both in the absence and in the presence of $\text{Cu}^{2+}$ ions by means of pH-metric (potentiometric) titrations (0.1 M Me$_4$NCl, 298.1 ± 0.1 K). Indeed, the potentiometric data, treated with the computer program HYPERQUAD [12], revealed that many protonated forms of L2 are able to bind both the $\text{SO}_4^{2−}$ anion alone and the $\text{Cu}^{2+}/\text{SO}_4^{2−}$ ion-pair. The equilibrium constants for the formation of $\text{SO}_4^{2−}$ complexes are reported in Table 3 (see Figure S3).
for a distribution diagram). This table includes the overall constants (β values) for the binding of SO₄²⁻ along with the constants for the equilibria by protonated ligand species (H₄L₂(−n)+ + SO₄²⁻ = [H₄L₂(SO₄)]⁽ⁿ⁻2⁾) that could be calculated from the former by using the ligand protonation constants [6]. It is to be noted that the constants for the latter equilibria could not be calculated for complexes [H₄L₂(SO₄)]⁽ⁿ⁻2⁾ with n < 11 (Table 3), since it was not possible to resolve as single proton binding processes the protonation equilibria involving H₄L₂⁽ⁿ⁺⁾ species with n < 11 [6]. Nonetheless, the stability constants that are available for the binding of SO₄²⁻ to the protonated ligand forms some peculiarities of this ligand. The stability of anion complexes of polyammonium ligands is generally determined by electrostatic attraction and hydrogen bonding [17–21]. Conversely, the ability of L₂ to bind SO₄²⁻ appears to be unaffected by its positive charge, that is, by its protonation state. Actually, the equilibrium constants for the anion binding vary in a very reduced range and their values are very small, on consideration of the high positive ligand charge and in comparison with SO₄²⁻ complexes of other polyammonium ligands [22]. A similar behaviour was also observed for phosphate and phosphate-like anion complexes with L₂, although, in several cases, the stability of these complexes was significantly higher, probably due to the greater hydrogen bond ability of phosphate-like anions [6]. Also the trend of stability is particular: the stability constants decrease from H₁₁L₂⁽¹⁺⁾ (logK = 3.10) to H₁₃L₂⁽³⁺⁾ (logK = 2.46), then steadily increase up to the formation of the complex with H₁₈L₂⁽¹⁸⁺⁾ (logK = 3.32).

| Equilibria                               | logK   | Equilibria                               | logK   |
|-----------------------------------------|--------|-----------------------------------------|--------|
| L₂ + 3H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁺             | 38.09 (5) |
| L₂ + 5H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽²⁺⁾         | 57.88 (5) |
| L₂ + 7H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽⁴⁺⁾         | 76.63 (5) |
| L₂ + 9H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽⁶⁺⁾         | 94.62 (5) |
| L₂ + 11H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽⁸⁺⁾         | 111.54 (5) |
| L₂ + 12H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹⁰⁺⁾        | 119.58 (5) |
| L₂ + 13H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹²⁺⁾        | 127.24 (5) |
| L₂ + 15H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹⁴⁺⁾        | 139.90 (5) |
| L₂ + 16H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹⁶⁺⁾        | 145.53 (5) |
| L₂ + 17H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹⁸⁺⁾        | 149.44 (5) |
| L₂ + 18H⁺ + SO₄²⁻ = [H₄L₂(SO₄)]⁽²⁰⁺⁾        | 152.12 (5) |
| H₁₁L₂⁽¹⁺⁾ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹⁺⁾         | 3.10 (7)  |
| H₁₂L₂⁽³⁺⁾ + SO₄²⁻ = [H₄L₂(SO₄)]⁽³⁺⁾         | 2.81 (7)  |
| H₁₃L₂⁽¹⁺⁾ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹⁺⁾         | 2.46 (7)  |
| H₁₄L₂⁽³⁺⁾ + SO₄²⁻ = [H₄L₂(SO₄)]⁽³⁺⁾         | 2.59 (7)  |
| H₁₅L₂⁽¹⁺⁾ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹⁺⁾         | 2.76 (7)  |
| H₁₆L₂⁽³⁺⁾ + SO₄²⁻ = [H₄L₂(SO₄)]⁽³⁺⁾         | 2.91 (7)  |
| H₁₇L₂⁽¹⁺⁾ + SO₄²⁻ = [H₄L₂(SO₄)]⁽¹⁺⁾         | 3.32 (7)  |

To get information about the possibility that such behaviour originates form the structural characteristics of the anion complexes, we performed a molecular modelling calculation on the [H₆L₂(SO₄)]⁽⁺⁴⁾, [H₁₂L₂(SO₄)]⁽¹⁺₁₀⁾ and [H₁₃L₂(SO₄)]⁽¹⁺₁₃⁾ species, assuming that the localization of H⁺ ions in the protonated ligand forms is as previously established by ¹H-NMR spectroscopy [6], that is, the first 12 H⁺ ions bind the 12 primary N(a) atoms (Figure 1), while in H₁₅L₂⁽¹⁵⁺⁾ the three additional protons involve the three tertiary N(c) nitrogen atoms. In [H₄L₂(SO₄)]⁽⁺⁴⁴⁾, protonation was assumed to occur on primary amine groups located as far apart as possible from each other. The minimum energy structures calculated for these complexes, reported in Figure 3, show that the ligand molecule becomes increasingly expanded while becoming increasingly protonated, as a consequence of the increasing electrostatic repulsion exerting between the ammonium groups. In the minimum energy structures of [H₄L₂(SO₄)]⁽⁺⁴⁾ (Figure 3a) and [H₁₂L₂(SO₄)]⁽¹⁺₁₀⁾ (Figure 3b), the SO₄²⁻ anion forms four salt-bridges (charge reinforced hydrogen bonds) with four ammonium groups of the ligand, while in [H₁₃L₂(SO₄)]⁽¹⁺₁₃⁾ (Figure 3c) such interactions drop to three and become longer. Most likely, the two opposite trends developing with increasing ligand protonation, namely (i) the favourable contribution due to the increasing ligand charge; (ii) the unfavourable contribution determined by ligand expansion, are responsible for the particular trend of complex stability showing a minimum for SO₄²⁻ binding by H₁₃L₂⁽¹⁺₃⁾ (logK = 2.46, Table 3).
The equilibrium constants determined for such complexes are presented in Table 4 in the form of each system, as a function of pH, in the form

...can make use of the so-called conditional (effective) stability constants that can be calculated for each system, as a function of pH, in the form

...increase in stability from log

...binding by species with equal positive charge, such as H

...species in lower protonation state than H

...protons on the ligand in the different species [23]. As can be seen from Figure 4, which shows the variation with pH of the effective stability constants calculated for SO

...presence of Cu2+ promotes the binding of SO42

...CuH9L2(SO4)19+. An assessment of the ability of the ligand to bind the anion in the absence or in the presence of Cu2+ ions can be performed by direct comparison of the equilibrium constants in Tables 3 and 4, limited to species with H

...this problem, we can make use of the so-called conditional (effective) stability constants that can be calculated for each system, as a function of pH, in the form

\[ K_{\text{eff}} = \Sigma[AH_iL]/(\Sigma[H_jL] \times [A]) \]

...for ion-pair complexes, where i, j, k and l are...
which shows the variation with pH of the effective stability constants calculated for \( \text{SO}_4^{2-} \) and ion-pair complexes, the presence of \( \text{Cu}^{2+} \) promotes the binding of \( \text{SO}_4^{2-} \) below pH 9, while in the range 9 < pH < 10.5 there is a preference for the metal-free ligand. This behaviour suggests the involvement of the metal ion in the binding of \( \text{SO}_4^{2-} \) in the ion-pair complexes of higher protonation state. At high pH values, the ligand is poorly protonated and thus it is able to fulfil the coordination sphere of \( \text{Cu}^{2+} \), preventing metal coordination to \( \text{SO}_4^{2-} \). The ligand wraps around the metal ion leaving less space for \( \text{SO}_4^{2-} \). Upon protonation of the \( \text{Cu}^{2+} \) complex, the ligand becomes less involved in the coordination to the metal and the increasing positive charge of the complex expands its structure, thus making space for the anion to get in contact with \( \text{Cu}^{2+} \) and form an increasing number of salt-bridges with ligand ammonium groups. At the break point of these trends (pH 9), the main ion-pair species in solution is \([\text{CuH}_7\text{L}_2(\text{SO}_4)]^{17+}\) (Figure S4). Below pH 6.5, the separation between the two curves in Figure 4, becomes about 2 logarithm units, which corresponds to the 11 kJ/mol free energy increment observed above for the binding of \( \text{SO}_4^{2-} \) to \( \text{CuH}_{18}\text{L}_2^{18+} \) relative to \( \text{H}_3\text{L}_2^{18+} \). The formation of contact ion-pair complexes was previously reported for the G-2 dendrimer L1, and is corroborated for L2 by the fact that the binuclear \( \text{Cu}_2\text{L}_2^{2+} \) complex binds \( \text{SO}_4^{2-} \) (Table 4) in the absence of ligand ammonium groups (ligand protonation).

### Table 4. Stability constants of the ion-pair complexes formed by L2 with \( \text{Cu}^{2+} \) and \( \text{SO}_4^{2-} \). 0.1 M \( \text{Me}_4\text{NCl} \), 298.1 ± 0.1 K. Values in parentheses are standard deviation on the last significant figure.

| Equilibria                                                      | log\(K\)  |
|---------------------------------------------------------------|------------|
| \( \text{CuH}_3\text{L}_2^{5+} + \text{SO}_4^{2-} = [\text{CuH}_3\text{L}_2(\text{SO}_4)]^{3+} \) | 3.10 (8)   |
| \( \text{CuH}_3\text{L}_2^{7+} + \text{SO}_4^{2-} = [\text{CuH}_3\text{L}_2(\text{SO}_4)]^{5+} \) | 3.33 (5)   |
| \( \text{CuH}_3\text{L}_2^{9+} + \text{SO}_4^{2-} = [\text{CuH}_3\text{L}_2(\text{SO}_4)]^{7+} \) | 3.51 (5)   |
| \( \text{CuH}_6\text{L}_2^{11+} + \text{SO}_4^{2-} = [\text{CuH}_6\text{L}_2(\text{SO}_4)]^{9+} \) | 3.62 (5)   |
| \( \text{CuH}_{10}\text{L}_2^{12+} + \text{SO}_4^{2-} = [\text{CuH}_{10}\text{L}_2(\text{SO}_4)]^{10+} \) | 3.44 (5)   |
| \( \text{CuH}_{11}\text{L}_2^{13+} + \text{SO}_4^{2-} = [\text{CuH}_{11}\text{L}_2(\text{SO}_4)]^{11+} \) | 3.69 (5)   |
| \( \text{CuH}_{12}\text{L}_2^{14+} + \text{SO}_4^{2-} = [\text{CuH}_{12}\text{L}_2(\text{SO}_4)]^{12+} \) | 3.96 (5)   |
| \( \text{CuH}_{13}\text{L}_2^{15+} + \text{SO}_4^{2-} = [\text{CuH}_{13}\text{L}_2(\text{SO}_4)]^{13+} \) | 4.31 (5)   |
| \( \text{CuH}_{14}\text{L}_2^{16+} + \text{SO}_4^{2-} = [\text{CuH}_{14}\text{L}_2(\text{SO}_4)]^{14+} \) | 4.64 (5)   |
| \( \text{CuH}_{16}\text{L}_2^{18+} + \text{SO}_4^{2-} = [\text{CuH}_{16}\text{L}_2(\text{SO}_4)]^{16+} \) | 5.20 (5)   |
| \([\text{CuH}_{16}\text{L}_2(\text{SO}_4)]^{16+} + \text{H}^+ = [\text{CuH}_{17}\text{L}_2(\text{SO}_4)]^{17+} \) | 2.78 (5)   |
| \( \text{Cu}_2\text{L}_2^{4+} + \text{SO}_4^{2-} = [\text{Cu}_2\text{L}_2(\text{SO}_4)]^{2+} \) | 4.01 (5)   |

**Figure 4.** Logarithms of the conditional stability constants of anion (\( \text{SO}_4^{2-} \)) and ion-pair (\( \text{Cu}^{2+}/\text{SO}_4^{2-} \)) complexes with L2.
3. Materials and Methods

3.1. General Information

All starting materials were high purity compounds purchased from commercial sources and used as supplied. Ligand L2 was synthetized according to a previously described procedure [24].

3.2. Potentiometric Measurements

Potentiometric (pH-metric) titrations, employed to determine equilibrium constants, were performed in 0.1 M Me₄NCl aqueous solution at 298.1 ± 0.1 K by using an automated system and a procedure already described [25]. The combined Metrohm 6.0262.100 electrode (Metrohm AG, Herisau, Switzerland) was calibrated as a hydrogen-ion concentration probe by titration of previously standardized amounts of HCl with CO₂-free NM₂OH solutions and determining the equivalent point by Gran’s method [26], which gives the standard potential, \( E^\circ \), and the ionic product of water \( pK_w = 13.83 \) (1) in 0.1 M Me₄NCl at 298.1 ± 0.1 K. The computer program HYPERQUAD [12] was used to calculate complex stability constants. All experiments were performed in the pH range 2.5–11.0 with \( 1 \times 10^{-3} \) M ligand concentration. Six titrations in the case of Cu²⁺ complexation, and five in the case of Zn²⁺, were performed with metal concentration varying in the ranges \( 0.5[L] \leq [Cu^{2+}] \leq 4.5[L] \) and \( 0.5[L] \leq [Zn^{2+}] \leq 3.5[L] \). Metal to ligand molar ratios greater than 5 for Cu²⁺ and 4 for Zn²⁺ were also tested: precipitation of metal hydroxide was observed in alkaline solution, while the analysis of the acidic branches of the titrations confirmed the maximum nuclearity of 5 for Cu²⁺ and 4 for Zn²⁺. Three titrations were performed for anion binding with SO₄²⁻ concentration in the range \( 2[L] \leq [SO_4^{2-}] \leq 5[L] \). Three titrations were performed for ion-pair binding with \( [Cu^{2+}] = 0.8[L] \) and SO₄²⁻ concentration \( 2[L] \leq [SO_4^{2-}] \leq 5[L] \). The different titration curves, obtained for metal, anion and ion-pair complexation experiments, respectively, were treated as separated curves without significant variations in the values of the common stability constants. Finally, the sets of data were merged together and treated simultaneously to give the final stability constants. Different equilibrium models for the complex systems were generated by eliminating and introducing different species. Only those models for which the HYPERQUAD program furnished a variance of residuals \( \sigma^2 \leq 9 \) were accepted. This condition was unambiguously met by a single model. Ligand protonation constants were taken from the literature [6].

3.3. Molecular Modelling

Molecular modelling investigations on \([H_6L_2(SO_4)]^{4+}\), \([H_{12}L_2(SO_4)]^{10+}\) and \([H_{15}L_2(SO_4)]^{13+}\) complexes were performed by means of the empirical force field method AMBER3 as implemented in the Hyperchem 7.51 package [27], using an implicit simulation of aqueous environment \((\varepsilon = 4 r)\) and atomic charged evaluated at the semiempirical level of theory (PM3) [28,29]. Potential energy surface of all the systems were explored by means of simulated annealing \((T = 600 K, \text{equilibration time} = 10 \; \text{ps}, \text{run time} = 10 \; \text{ps} \; \text{and} \; \text{cooling time} = 10 \; \text{ps}, \text{time step} = 1.0 \; \text{fs})\). For each studied system, 80 conformations were sampled.

As for the Zn(II) complexes, the trinuclear Zn₃L₂⁶⁺ species was firstly analysed. Starting coordinates were built from the crystal structure of the Ni²⁺ complex of L₁ [1], containing Ni²⁺ ions hexacoordinated in distorted octahedral environments to six out of the seven nitrogen atoms constituting a portion of L₁ that is identical to the three branches of L₂ growing from the central N(c) atom (Figure 1). The nitrogen atom remaining uncoordinated is a primary one. This structural motif was chosen taking into account that, according to the equilibrium data discussed before, all three metal ions of Zn₃L₂⁶⁺ should be hexacoordinated and three primary amine groups of the complex should not be involved in metal coordination. This crystallographic structural unit was firstly modified by replacing Ni²⁺ with Zn²⁺ and completing each coordination environment with water molecules.

The starting coordinates for the binuclear Zn₂L₂⁴⁺ complex were obtained by deleting one zinc ion in the QM minimized structure of Zn₃L₂⁶⁺. The tetranuclear Zn₄L₂⁸⁺ complex was instead obtained
from the QM minimized binuclear complex by adding to its metal-free branch two QM minimized pentacoordinated Zn$^{2+}$ complexes, one in square pyramidal and one in bipyramidal geometry, taken from the crystallographic structure of the trinuclear Cu$^{2+}$ complex of L1 [1] and successively modified by replacing Cu$^{2+}$ with Zn$^{2+}$.

The starting coordinates for each polynuclear complexes were firstly optimized by using the OPLS2005 forcefield implemented in the Impact software [30], with completely frozen metals and coordination environments. Then, each MM minimized structure was fully optimized at the DFT/M06 level of theory [31,32] by using the 6–31 g(tm) basis set [33–37] and the implicit simulation for the aqueous environment [38]. The nature of stationary points as true minima was checked by frequency calculations.

4. Conclusions

The ability of L2 to form stable highly nucleated complexes over a large pH range, as a consequence of its dendrimeric nature and of the many amine groups in its structure, make this compound a promising candidate for the preparation of solid supported materials to be used in the recovery of metal ions from aqueous media. This could find applications in both decontamination of waste waters and in the extraction of precious metals. Indeed, it was recently reported that activated carbon functionalized with randomly structured poly(ethylene imine) dendrimers are efficient scavenger of Pd$^{2+}$ cations [39]. Moreover, L2 is also a promising candidate for catalytic purposes. The use of molecules with well-defined molecular structures, such as L2, has the advantage that with such molecules it is possible to perform a confident speciation of the complexes they form in solution, thus getting a fundamental instrument for the tailoring of appropriate receptors for substrates binding and activation. This is of special interest when the supported complex is used for catalytic purposes. Considering the ability of L2 to form complexes with many metal centres that may promote the binding of further species from the medium, we are particularly interested in developing carbon materials (activated carbons, carbon nanotubes, graphene) functionalized with L2 and testing them for catalytic applications in reaction for the formation of carbon-carbon bonds, such as the Sonogashira cross coupling.

Supplementary Materials: The following are available online: Figure S1: Distribution diagrams of the Cu$^{2+}$ complexes of L2, Figure S2: Distribution diagrams of the Zn$^{2+}$ complexes of L2, Figure S3: Distribution diagrams of the anion complexes formed by L2 with SO$^{42-}$, Figure S4: Distribution diagrams of the ion-pair complexes formed by L2 with Cu$^{2+}$ and SO$^{42-}$ complexes of L2.

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Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of the compound L2 is available from the authors.