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Binding constant determination of WIN 22169, a novel polymeric ligand

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Abstract: WIN 22169 is a co-polymer containing approximately 11 repeating units of polyoxyethylene and diethylenetriamine pentaacetic acid (DTPA). WIN 66368, a magnetic resonance imaging (MRI) contrast agent, is the gadolinium III complex of WIN 22169. WIN 22169 has been characterized with respect to its equivalent weight, acidity constants and excess acid or base, as well as its metal ion binding constants. The logs of the equilibrium binding constants of the ligand to Gd^{3+}, Ca^{2+}, Zn^{2+} and Cu^{2+} were found to be 16.6, 7.47, 12.2 and 14.0. The Gd selectivity constant, a measure of the preferential binding of the ligand toward Gd^{3+} versus the three *in vivo* ions: Ca^{2+}, Zn^{2+} and Cu^{2+}, of WIN 66368 was calculated to be 7.9. This value compares favourably to that for Gd DTPA which has a Gd selectivity constant of 7.04.

Keywords: *HPLC, potentiometric titration; binding constant; formation constant; stability constant; gadolinium; MRI contrast agent.*

Introduction

Diethylenetriamine pentaacetic acid (DTPA) can be co-polymerized with polyoxyethylene, bearing amine terminating groups, through the formation of amide bonds (Structure 1). The resulting novel polymeric ligand, WIN 22169, forms a paramagnetic complex with Gd^{3+}, WIN 66368, useful as a constant agent for MRI. Rocklage *et al.* have shown that the toxicities of Gd chelates, such as GdDTPA BMA and Gd DTPA, the active ingredients in Omniscan® and Magnevist®, respectively, are related to the complex binding constant for ligand to Gd taken together with similar constants for the *in vivo* metals, calcium, zinc, and copper [1]. It is therefore desirable during the preformulation phase of drug development to measure the binding constants of MRI ligands in order to estimate the potential toxicity of the agent and to rationalize the toxicity test data obtained.

Good estimates of both the equivalent weight and the excess acid or base present in the drug substance, that is, the deviation from exact neutralization, are required for the calculation of ligand pK_as and metal binding constants. Although the average molecular weight of the polymeric ligand can be measured, this datum does not yield an accurate value for the equivalent weight with respect to metal due to uncertainty about chain branching and the
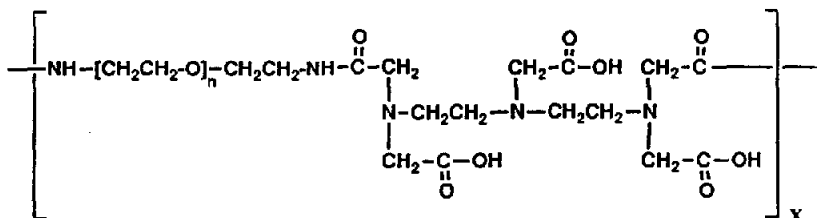
Structure 1
Chemical structure of WIN 22169 where n is 32 on average and X is 11 on average.

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nature of the end groups of the polymer. Exact neutralization of the acid functions, after synthesis of the ligand, is generally not practical, but is not needed for the further synthesis of the paramagnetic complex.

In this study, the equivalent weight of the polymeric ligand was determined by titrating it with a standard calcium solution; a calcium-selective electrode was used to detect the end point. The excess acid or base associated with the ligand was analysed by a potentiometric pH titration. The Gd binding constant was measured by ligand competition, using 2,6-bis(aminomethyl)pyridinetetraacetic acid (PBMNTA) as the gauge ligand [2]. Binding constants for the less strongly held metals, Ca^{2+}, Zn^{2+} and Cu^{2+}, were determined by potentiometric pH titration. The toxicity of the complex was estimated by means of the Rocklage relationship [1].

Materials and Methods

Reagents

WIN 22169, WIN 66368 and 2,6-bis(aminomethyl)pyridinetetraacetic acid disodium salt (PBMNTA) were provided by the Medicinal Chemistry Department, Sterling Winthrop Pharmaceuticals Research Division. Size exclusion HPLC showed WIN 22169 to have a weight average molecular weight of 14,900; an average molecular weight of 8300; and a polymer dispersity of 1.70.

Ethylenediaminetetraacetic acid (EDTA) disodium salt, volumetric standard, 49.9 mM; tris(hydroxymethyl)aminomethane (Tris), ACS reagent grade; potassium hydrogen phthalate, ACS primary standard; potassium chloride; copper, volumetric standard (0.198 mg Cu/ml); zinc sulphate, volumetric standard (0.0499 M); calcium chloride dihydrate, ACS reagent; were obtained from Aldrich Chemical Co. (Milwaukee, WI). Potassium hydroxide 0.1 N Dilut-It analytical concentrate and hydrogen chloride 0.1 N, Dilut-It analytical concentrate were obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). Purified water was used throughout the study.

pK_a and equivalent weight determination

All titrations were carried out by means of a Radiometer ABU93 automatic buret controlled by a Radiometer VIT90 titrator (Radiometer Analytical Instruments, Copenhagen,

Denmark). A Radiometer pH electrode and a single junction silver–silver chloride reference electrode (Fisher Scientific, Pittsburgh, PA) were used. The titration vessel was thermostated at 25 ± 0.2°C, and was continuously purged with argon gas. The titration data were transferred to a PC computer for processing. All titrations were carried out in triplicate; agreement between the replicates was excellent and the average values were used. The relative standard deviation of the measured pH in the buffer region of the titration curve was less than 3.0%, except for the end point region where the standard deviation was as high as 12%.

A 0.1 N KOH solution was prepared from distilled water, which had been boiled for 30 min to remove carbon dioxide and allowed to cool under the protection of a soda lime trap. J.T. Baker Dilut-It concentrate was the source of KOH. A Gran regression plot established that the solution contained a negligible concentration of carbonate [3]. The solution was standardized against potassium hydrogen phthalate. The end point was established by means of the appropriate Gran regression (weak acid, strong base) [4]. The average of three replicate titrations was 0.0998 N with a coefficient of variation of 0.4%. The base titrant was protected with a continuous blanket of argon (Union Carbide Corp., Linde prepurified grade). Dilut-It 0.1 N HCl was standardized with the base, and was found to be 0.0996 N.

Following Martell and Motekaitis [3], acidities were represented by p[H], the negative logarithm of the hydrogen ion concentration, rather than by the measure of activity, pH. Buffers of known activity were used to calibrate the pH meter, which was subsequently used to measure a series of solutions of known hydrogen ion concentration, generated by titrating HCl with KOH, in the presence of KCl at experimental concentrations. The pH reading of the meter was corrected to p[H] by adding the difference between the concentration calculated from the volumes of the titrants added and the pH reading. The correction term was −0.045 (p[H] − pH). A dissociation constant for water, pK_a, equal to 13.90 was required to make the correction term (above) calculated in the acidic region equal to that calculated in the basic region. This datum can be considered the dissociation constant for water under the conditions of the experiments.

A solution of WIN 22169 was prepared in 0.1 M KCl. To 20 ml of this solution, which contained 0.0171 mM of ligand, was added 1 ml (0.0.0996 mEq) of the HCl titrant, and the resulting solution titrated with the KOH titrant. The method of calculation of pK_a is described in the Results section.

A stock solution of calcium chloride (0.0531 M), prepared from calcium chloride dihydrate, was titrated against ethylene-diaminetetraacetic acid, disodium salt, volumetric standard (0.0499 M) using a calcium selective electrode (Orion Research Inc., Boston, MA) to detect the end point. The standardization procedure was as follows. To the titration vessel was added 25 ml of 0.1 M KCl in 0.001 N KOH, 1 ml of EDTA volumetric standard and 0.75 ml of 0.1 M KOH to adjust the pH to 10.9. This solution was titrated with calcium chloride. The end point was determined by a Gran regression. A stock solution of WIN 22169, lot XA, was prepared in 0.1 M KCl at a concentration of 3.963 mg ml^{-1} and lot XD at 4.158 mg ml^{-1}. For the determination of equivalent weight, a 20 ml aliquot was adjusted to a pH of between 10.8 and 10.9 and titrated with CaCl$_2$ (0.0531 M).

Determination of Ca^{2+}, Zn^{2+} and Cu^{2+} binding constants

The binding constants of WIN 22169 with Ca^{2+}, Zn^{2+} and Cu^{2+} ions were determined by potentiometric (pH) titration at 25°C. These chelates were formed *in situ* as follows. A primary stock solution of CaCl$_2$ was prepared in water at a concentration of 0.01036 M. The Ca chelate of lot XA was prepared by diluting 1.26 g of WIN 22169 with 50 ml of CaCl$_2$ primary stock (0.01036 M) and 0.75 g of KCl to 100 ml mark with water. The Zn chelate of lot XA was prepared by diluting 1.2 g of WIN 22169, 10 ml of Zn sulphate volumetric standard (0.0499 M) and 0.75 g of KCl to 100 ml. The Cu chelate was prepared by diluting 86.6 mg of WIN 22169, 11.6 ml of CuSO$_4$ volumetric standard (0.198 g Cu ml^{-1}) and 0.37 g of KCl to 50 ml with water. To the titration vessel was added 15 ml of chelate solution. For Ca and Zn, the titration vessel was pre-dosed with 1.0 ml of 0.1 N HCl. Cu and Zn chelates were titrated with 0.1 ml increments of 0.1 N KOH whereas the Cu chelate was titrated with 0.03 ml increments of 0.1 N KOH.

Determination of the Gd^{3+} binding constant

Stock solutions of Tris HCl (0.111 M), prepared with HCl and Tris (0.111 M), and Tris (0.111) containing 0.111 M KCl, were prepared in water. From these two solutions, a Tris buffer was prepared at pH 9.7. A PBMNTA stock solution was prepared at a concentration of 16.10 mM in water. A WIN 66368 stock solution was prepared at a concentration of 3.131 mM containing 0.1 M KCl. Competition solutions containing PBMNTA (0.785 mM) and WIN 66368 (0.783 mM) were prepared from a 0.4 ml aliquot of PBMNTA (16.10 mM), 3.6 ml of Tris buffer, 2.0 ml of WIN 66368 (16.10 mM), and 2.0 ml of 0.1 M KCl. A second set of competition solutions containing PBMNTA (0.785 mM) and WIN 66368 (0.587 mM) was prepared from a 0.4 ml aliquot of PBMNTA (16.10 mM), 3.6 ml of Tris buffer, 1.5 ml of WIN 66368 (3.131 mM), and 2.0 ml of 0.1 M KCl. A third set of competition solutions containing PBMNTA (0.785 mM) and WIN 66368 (0.391 mM) was prepared from a 0.4 ml aliquot of PBMNTA (16.10 mM), 3.6 ml of Tris buffer, 1.0 ml of WIN 66368 (3.131 mM), and 2.5 ml of 0.1 M KCl. Competition solutions were prepared in triplicate and equilibrated for 5 days at 25°C.

Samples were assayed without dilution by an HPLC method previously described [2, 5]. A Waters HPLC system was used, which consisted of a 510 solvent delivery system, a 486 UV–vis detector, a Maxima 820 data module, and a 700 Satellite WISP. Samples were separated on a PRP-X100 stainless steel column, 150 × 4.1 mm (Hamilton Co., Reno, NV). The mobile phase consisted of 0.1 M Tris, 0.025 M KCl, and 1 mM EDTA tetrasodium salt adjusted to pH 8.0 with concentrated HCl. The injection volume was 15 µl. Detection was at 272 nm. The flow rate was variable, increasing linearly from 1 to 4.0 ml min^{-1} at 7.0 min. Under these conditions the retention time of Gd PBMNTA and PBMNTA were 2.7 and 8.0 min, respectively. Linear calibration plots were prepared from peak responses of at least four standards each of GdPBMNTA and PBMNTA. Standards were prepared in 0.05 M Tris.

Results and Discussion

The number of equivalents and the equivalent weights of two lots (XA and XD) of WIN 22169 were determined by titration with a

standard calcium chloride solution. The voltage response of a calcium-selective electrode was recorded as a function of the volume of calcium solution added during titration. The end point, at which the number of equivalents of WIN 22169 was equal to the number of equivalents of calcium added, was calculated by means of a Gran regression of the data after the end point [4]. The sensitivity of the calcium electrode was not sufficient to quantify the low calcium concentrations present before the end point, when the ligand was in excess. In accordance with Gran's method, the quantity, $(V_0 + V)\ 10^{17n(E-K)}$, was a linear function of V. V_0 is the original volume of WIN 22169 solution; V, the volume of calcium solution added; n, the number of electrons (2) contributing to the potential; E, the potential in volts; and K, an arbitrary constant. The intercept of the regression divided by the slope gives the end point volume. A typical titration curve with the Gran plot superimposed is

shown in Fig. 1. The number of equivalents and derived equivalent weights of ligand are in Table 1. The number of equivalents being equal to the number of available chelating sites.

In order to determine the excess acid or base associated with the ligand, a known volume of standard acid was added to a solution of the ligand, and a pH titration carried out with standard base. The acidity constants of the ligand do not fall within a range appropriate for the use of a Gran plot (Gran II method) for the detection of the end point [6]. The computer program, BEST, with slight modification, was used to calculate the excess acid or base from the complete titration curve [3]. To fit a calculated to an experimental titration curve, BEST varies the pK_a values. However, optionally, the concentrations of selected components can also be varied to improve the fit. In order to determine the excess acid or base present in drug substance, BEST was allowed
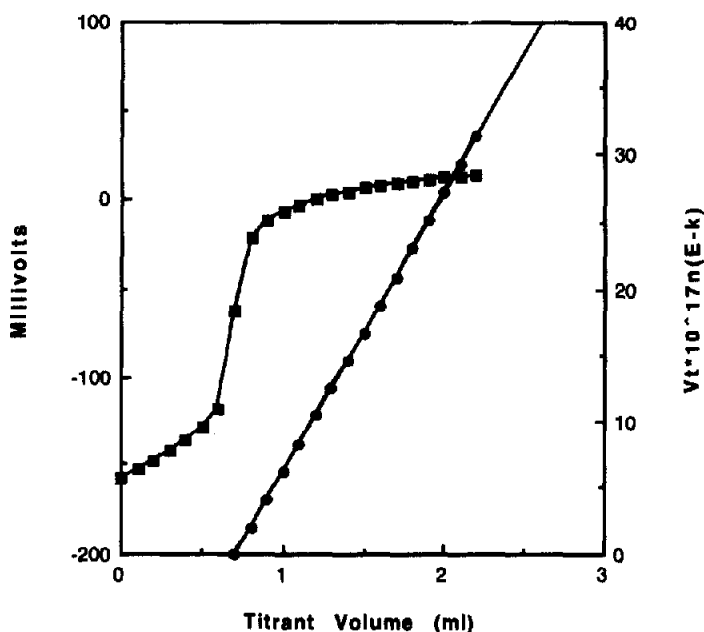
Figure 1
Calcium titration of WIN 22169 (lot XD) using a calcium selective electrode: ■, represents titration data and ●, Gran regression.

Table 1
Equivalent weight determination of WIN 22169 by potentiometric calcium titration with $CaCl_2$ (53.13 mM)

Lot	End point (ml)	SD (ml)	mEq Ca^{2+}	Ligand (mg)	Eq. weight
XA	0.6232	0.0028	0.0331	79.26	2395
XD	0.7059	0.0014	0.0375	83.16	2217

to vary the total concentration of ionizable hydrogen to improve the fit. This has the effect of translating the calculated titration curve along the titrant volume axis. Normally, BEST de-weights the titration data near an end point, because the solution in that region is not well buffered; the pH is unstable and changing rapidly, so the pH data are less reliable. The data near the end point are nevertheless indispensable for establishing the translation of the pH curve along the titrant volume axis. The data weighting factors of the BEST program were removed for calculations of excess acid or base. The equivalents of excess acid or base were calculated by subtracting the equivalents of acid added prior to titration from the equivalents of acid determined by the modified BEST program to give the best fit of the calculated to the experimental curve. This number was further reduced by three times the equivalents of ligand present based on the calcium titration (above), three being the number of titratable protons at each calcium chelating site on the ligand. The equivalent percentage of acid, or in the case of lot XD, base, is expressed by (100 × equivalents excess acid)/(3 × equivalents of ligand). Lot XA was found to have 2.2% excess acid; whereas, lot XD was found to have 3.0% excess base. The final pK_as were calculated with the unmodified BEST program, using the values determined above for excess acid and for the equivalent

weight; these data are listed in Table 2. Following the advice of Martell and Motekaitis [3], the pH meter was calibrated with strong acid solutions in order to produce hydrogen ion concentration data, expressed as the negative logarithm, p[H]. The titration curve is shown in Fig. 2.

The binding constants for Ca^{2+}, Zn^{2+} and Cu^{2+} were sufficiently low so that they could be determined by direct potentiometric titration of solutions containing WIN 22169 (lot XA). After the addition of an initial volume of strong acid, the solutions were titrated with base, and the pH recorded. The titration data were analysed by means of the computer program, BEST [3]. From the known *total* concentrations of ligand and metal, the pK_as for the ligand determined (above), and a set of trial binding constants, the program solves for p[H] as a function of base added. The three non-linear equations, which must be solved, are below (illustrated for Ca):

$$T_L = [L3-] + K_{HL}[H^+][L^{3-}]$$
$$+ K_{HL}K_{H2L}[H^+]^2[L^{3-}]$$
$$+ K_{HL}K_{H2L}K_{H3L}[H^+]^3[L^{3-}]$$
$$+ K_{CaL}[M^{2+}][L^{3-}]$$
$$+ K_{CaL}K_{CaHL}[M^{2+}][H^+][L^{3-}] \qquad (1)$$

$$T_{Ca} = [Ca^{2+}] + K_{CaL}[Ca^{2+}][L^{3-}]$$
$$+ K_{CaL}K_{CaHL}[Ca^{2+}][H^+][L^{3-}] \qquad (2)$$

Table 2
Protonation constants and metal chelate binding constants [25°C, μ = 0.1 (KCl)]

		Log K				
		WIN 22169* lot				
Constant	Equilibrium	(XA)	(XD)	PBMNTA†	DTPA BMA‡	DTPA‡
K_{HL}	[HL]/[L][H]	9.14	9.0	8.68	9.37	10.49
K_{H2L}	[H₂L]/[HL][H]	4.71	4.75	8.15	4.38	8.60
K_{H3L}	[H₂L]/[H₂L][H]	3.96	4.03	2.62	3.31	4.28
K_{H4L}	[H₄L]/[H₃L][H]			2.10	1.43	2.64
K_{GdL}	[GdL]/[Gd][L]	16.6		18.6	16.85	22.46
K_{CaL}	[CaL]/[Ca][L]		7.47		7.17	10.75
K_{CaLH}	[CaHL]/[CaL][H]		4.85		4.45	6.11
K_{ZnL}	[ZnL]/[Zn][L]		12.2		12.04	18.7
K_{ZnLH}	[ZnHL]/[ZnL][H]		4.21		4.04	5.60
K_{CuL}	[CuL]/[Cu][L]		14.0		13.03	21.38
K_{cuLH}	[CuHL]/[CuL][H]		3.73		3.36	4.81
LD₅₀ (mmole kg⁻¹)§			10.0		14.8	5.6

*This work.
†Ref. 2.
‡Ref. 1.
§Predicted values calculated using the correlation reported by Rocklage and co-workers (1).
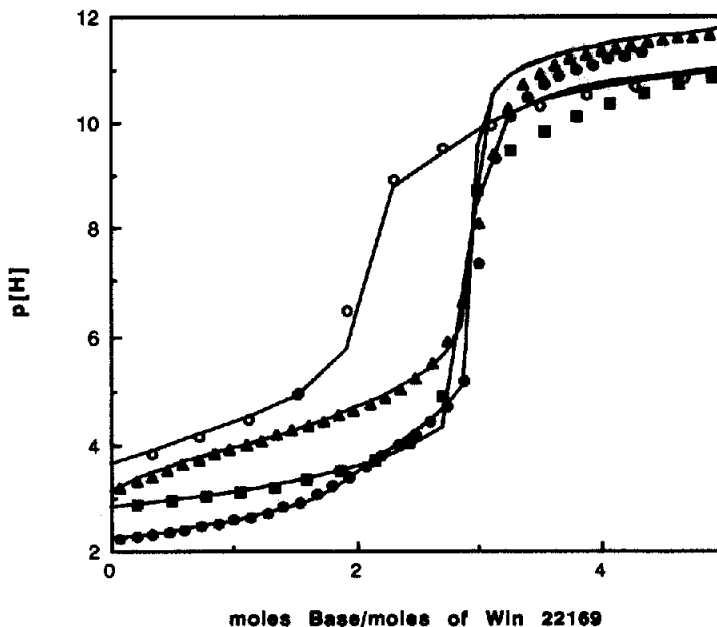
Figure 2
Potentiometric titration of WIN 22169 (lot XA) in the presence of various metal ions at 25°C. WIN 22169 in absence of metal ion (O), in presence of Ca (▲), Zn (●), and Cu (■). Symbols represent individual experimental data and solid lines the calculated curves.

$$T_H = K_{HL}[H^+][L^{3-}] + 2K_{HL}K_{H2L}[H^+]^2[L^{3-}] + 3K_{HL}K_{H2L}K_{H3L}[H^+]^3[L^{3-}] + [\text{Base added}] + [H^+] - K_{H_2O}[H^+]^{-1} + K_{CaL}K_{CaHL}[M^{2+}][H^+][L^{3-}] \quad (3)$$

in which T_L, T_{Ca} and T_H are the total concentrations of ligand, Ca, and hydrogen ion. The equilibrium constant nomenclature is explicated in Table 2. The program BEST compares the experimental titration curve to the calculated one, adjusts the equilibrium constants in an attempt to reduce the difference between the calculated and the experimental curves, recalculates the curve, and continues the process until no further improvement results. The binding constants found are listed in Table 2. A typical titration curve, with the calculated curve, is shown in Fig. 2. Beyond the equivalence point, the experimental curve lies below the calculated; at higher p[H] values formation of hydroxy-metal species might be expected. These species were not accounted for in the calculation; if present, they would cause a reduction in the p[H] below the expected value. In any case, the deviations occur at p[H] values above those at which the hydrogen ion, metal ion, exchange is occurring, and are thus not relevant to the metal ligand equilibrium constant measurement.

The affinity between WIN 22169 and Gd^{2+} is so strong that no significant replacement of metal by hydrogen ion occurs at accessible p[H] values and direct potentiometric titration is not feasible. Consequently, the constant was determined by ligand competition, using 2,6-bis(aminomethyl)pyridinetetraacetate (PBMNTA) as the competitor or gauge ligand. The method has been described [2]. Equation (4) represents the equilibrium exchange of Gd^{3+} between WIN 22169 and PBMNTA; equation (5) represents the composite equilibrium constant for the exchange. Equation (6) represents the binding constant to be determined; equation (7) the known equilibrium constant for PBMNTA.

$$Gd\,DTPA\,PEG + PBMNTA \rightleftharpoons Gd\,PBMNTA + DTPA\,PEG \quad (4)$$

$$K_3 = [Gd\,PBMNTA]\,[DTPA\,PEG]/[Gd\,DTPA\,PEG]\,[PBMNTA] \quad (5)$$

$$K_1 = [Gd\,DTPA\,PEG]/[Gd]\,[DTPA\,PEG] \quad (6)$$

$$K_2 = [Gd\,PBMNTA]/[Gd]\,[PBMNTA].\quad(7)$$

Since K_2 has previously been determined (log

K of 18.6 [2]), K_1 can be simply calculated from equation (8), using the HPLC method previously reported to measure the ratio of equation (5).

$$K_1 = K_2/K_3. \qquad (8)$$

The HPLC method measures the concentration of the GdPBMNTA complex and the *total* concentration of uncomplexed PBMNTA, which includes all of its protonated forms. The concentration of WIN 66368 and the total concentration of WIN 22169, which includes all of its protonated forms, can be calculated by difference, if it is assumed that the concentration of free Gd is negligible, since the initial concentration of WIN 66368 is known. If the acidity constants are known, [PBMNTA] and [WIN 22169] can be calculated from the total concentrations, $[PBMNTA]_T$ and $[WIN 22169]_T$, and the p[H]. This calculation is illustrated in general for a ligand, L; it follows the development of Rocklage [1]. Let the acidity constants be defined as; $K_{H1} = [HL]/[H][L]$, $K_{H2} = [H_2L]/[H][HL] = [H_2L]/K_{H1}[H]^2[L]$, etc. The ligand concentration determined from HPLC, L_T, is then given by equation (9) and the concentration of the deprotonated ligand by equation (10), [H] being fixed by the buffer.

$$L_T = [L] + [HL] + [H_2L] + \ldots \qquad (9)$$

$$L_T = [L](1 + K_{H1}[H] + K_{H1}K_{H2}[H]^2 + \ldots)$$
$$[L] = L_T/(1 + K_{H1}[H] + K_{H1}K_{H2}[H]^2 + \ldots). \qquad (10)$$

The acidity constants used in the calculations are listed in Table 2, and the binding constants in Table 3.

Free Gd III ion is toxic; this toxicity is a major concern in the development of Gd-based MRI agents. In studying the toxicity of Gd chelates, Rocklage and co-workers observed a poor correlation between the Gd binding constant and the toxicity of the chelates. Instead, they saw an excellent correlation between toxicity and a parameter they referred to as the 'selectivity constant' [1]. The selectivity constant incorporates not only the binding constant for Gd but also binding constants for complexation with Ca^{2+}, Zn^{2+}, and Cu^{2+}. This selectivity constant is a measure of the equilibrium exchange between the Gd ion in the chelate with endogenous Ca, Zn and Cu ions.

Table 3
Determination of the Gd³⁺ binding constant of WIN 22169

Initial [GdDTPA PEG] (mM)	Initial [PBMNTA] (mM)	Exp. [GdPBMNTA] (mM)	Exp. [PBMNTA]t (mM)	pH	Calc. [DTPA PEG] (mM)	Calc. [PBMNTA] (mM)	Log K_3 (mM)	Log K_1 (mM)
0.783	0.785	0.716	0.069	9.7	0.535	0.063	1.959	16.6
0.587	0.785	0.575	0.235	9.7	0.429	0.214	1.983	16.6
0.391	0.785	0.388	0.409	9.7	0.290	0.372	2.003	16.6

They conclude that the transmetalation exchange with these metal ions is the major cause of the release of free Gd^{3+} ions and thus the toxicity. K selectivity (K_{SEL}) is defined by

$$K_{SEL} = K_{therm}/(\alpha_H + \alpha_{Ca} + \alpha_{Zn} + \alpha_{Ca}) \quad (11)$$

$$\alpha H = 1 + K_{H1}[H^+] + K_{H1}K_{H2}[H^+]^2 + \ldots \quad (12)$$

$$\alpha_{Ca} = K_{CaL}[Ca^{2+}] \quad (13)$$

$$\alpha_{Zn} = K_{ZnL}[Zn^{2+}] \quad (14)$$

$$\alpha_{Cu} = K_{CuL}[Cu^{2+}]. \quad (15)$$

The concentrations of Ca^{2+}, Zn^{2+} and Cu^{2+} used in the calculation were 2.5, 0.050 and 0.001 mM, respectively, which, according to Rocklage [1], correspond to the average *in vivo* concentrations of these metals. Rocklage and co-workers found a linear relationship between log K_{SEL} of a variety of Gd chelates (GdDTPA, GdEDTA, GdDTPA BMA and GdDTPA-BP) and the LD_{50} (mmole kg^{-1}) determined in Swiss–Webster mice. For GdDTPA BMA, log K_{SEL} was 9.04, which, when using the linear relationship reported by Rocklage and co-workers, correlates with an LD_{50} of 14.8 mmol kg^{-1}. For GdDTPA, the respective values were 7.04 and 5.6 mmol/kg [1]. The constants reported in Table 2 for WIN 22169 lead to a log K_{SEL} of 7.9, which would predict an LD_{50} near 10 mmol kg^{-1}, between that of GdDTPA BMA and GdDTPA. Of course, as Rocklage has pointed out [1], the toxicity of a drug product also depends on its formulation, and might be reduced, for instance, by adding free ligand, assuming the ligand is not itself toxic.

Conclusions

In this study, potentiometric titration was used in combination with HPLC to characterize the metal binding properties of WIN 22169, a novel polymeric ligand. As a polymeric ligand, each WIN 22169 molecule contains multiple metal ion chelating groups. The reactivity of these groups could vary depending on their position within the polymer chain, implying a multiplicity of acidity and binding constants. In analysing the pK_as and binding constants, the simplifying approximation was made that there were only three distinguishable classes of titratable protons. The agreement of the experimental titration curve with the calculated one (Fig. 2) indicates that the approximation is a good one. The predicted LD_{50} value, using the correlation reported by Rocklage and co-workers, suggests the gadolinium toxicity of Win 22169 to be between that of GdDTPA and GdDTPA BMA.

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