Determination of the Stability Constants of Macrocyclic Ligand–Alkali Cation Complexes by Fast Atom Bombardment Mass Spectrometry

G. BONAS, C. BOSSO, and M. R. VIGNON*

Centre de Recherches sur les Macromolécules Végétales, CNRS, B.P. 53 X, 38041 Grenoble, France.

Abstract. Quantitative studies of the complexation of the macrocyclic ligands, 18 crown 6 (18C6, **1**) and cyclogentiotetraose peracetate (CGD4Ac, **2**), with alkali cations, have been investigated by fast atom bombardment mass spectrometry (f.a.b.m.s.). Complexation curves of 18C6 with Na^+, K^+, Rb^+ and Cs^+ in glycerol, and of CGD4Ac with Cs^+ in PEG 200, were obtained by plotting the complex peak intensity against the alkali cation concentration. From these curves we describe a method to calculate the stability constant for an alkali cation–macrocyclic ligand complex. There is a good agreement between stability constants obtained either by f.a.b.m.s. or calorimetric techniques for 18C6-alkali cation complexes. These results suggest that the f.a.b. technique can be used to study complex formation and determine the stability constant.

Key words. 18C6 crown ether, cyclogentiotetraose peracetate, alkali cations, complexation, stability constants, fast atom bombardment mass spectrometry.

1. Introduction

Quantitative aspects of fast atom bombardment mass spectrometry (f.a.b.m.s.). were investigated by Beckner and Caprioli [1] for several compounds of biological interest by using two types of internal standards. Caprioli also determined dissociation constants of weak acids in solution [2]. Johnstone *et al.* [3–5] studied the complex formation in aqueous glycerol and other solutions between crown ethers and metallic cations by f.a.b.m.s. The abundances of gas-phase ions at *m/z* values corresponding to metal cation–ligand complexes closely reflected the calculated concentrations of these complexes in solution. They drew complexation curves and discussed the energy transfer mechanisms. Their results suggested that metal cation-ligand complex formation in solution could be assessed rapidly or semi-quantitatively by the f.a.b. technique.

In this paper we report on quantitative studies of 18C6–alkali cation complexation; stability constants were determined by f.a.b.m.s. in glycerol. The same approach allowed the study of the complexation of cyclogentiotetraose peracetate with a cesium cation, in PEG 200. Stability constants in the PEG matrix with cesium chloride were calculated for each of the ligands 18C6, **1** and CGD4Ac, **2** and the relative affinity of Cs^+ for 18C6 and CGD4Ac is discussed.

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* Author for correspondence.
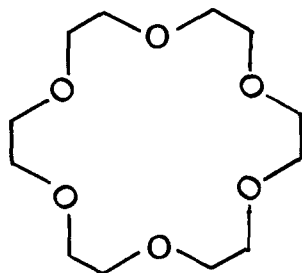
18 Crown 6

1
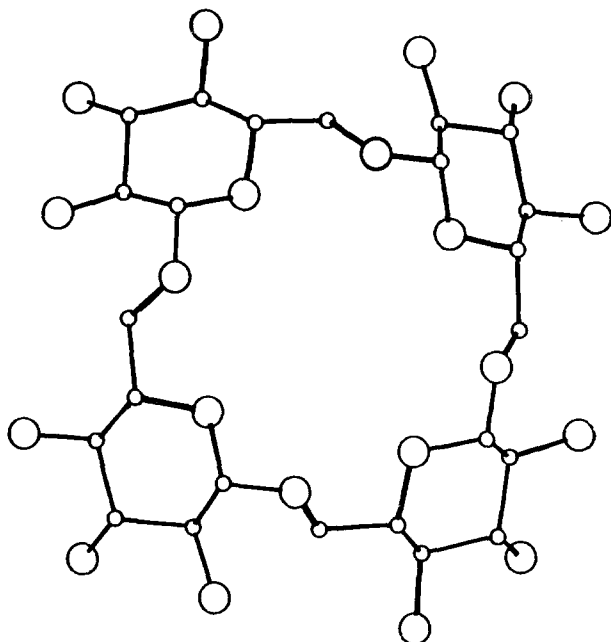
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2. Experimental

Fast atom bombardment mass spectra in the positive mode were recorded on a Nermag R 1010C apparatus equipped with an M Scan (Wallis) atom gun (8 kV; 20 μA).

The $(18C6-M)^+$ complex peak intensities were normalized to the glycerol matrix peak at $m/z = 185$ which is set at 100%. The $(CGD4Ac-M)^+$ complex peak intensities were normalized to the polyethylene glycol 200 (PEG 200) matrix peak at $m/z = 151$ or $m/z = 239$ which are set at 100%.

For measurements required for Figure 2, mass spectra were recorded after successive additions of 18C6 solution ($2 \mu L$; 5×10^{-5} M in water) on the f.a.b. probe containing $1 \mu L$ of KCl solution (2×10^{-2} M in water) and $5 \mu L$ of PEG 200 matrix.

In order to obtain the 18C6 complexation curves (Figure 3), the mass spectra were recorded after successive addition of $x \mu L$ of alkali chloride aqueous solution (A) on the f.a.b. probe containing $1 \mu L$ of 18C6 solution (5×10^{-4} M in water) and $5 \mu L$ of PEG 200 matrix:

- Cesium: $A_{Cs} = 2.5 \times 10^{-3}$ M in water; $x = 0, 1, 1, 2, 2, 2, 2, 3, 3, 3, 3, 3 \mu L$. Observed peak: $m/z = 397$.
- Rubidium: $A_{Rb} = 2.5 \times 10^{-4}$ M in water; $x = 0, 1, 1, 1, 1, 2, 2, 3, 3, 3, 3, 3, 3, 3, 3 \mu L$. Observed peak: $m/z = 349$.
- Potassium: $A_K = 10^{-4}$ M in water; $x = 0, 1, 1, 1, 1, 1, 1, 1, 3, 3, 4 \mu L$. Observed peak: $m/z = 303$.
- Sodium: $A_{Na} = 2.5 \times 10^{-3}$ M in water; $x = 0, 1, 1, 1, 1, 2, 2, 3, 3, 3, 3 \mu L$. Observed peak: $m/z = 287$.

The data required for Figure 4 were obtained by recording the mass spectra after successive additions of CGD4Ac solution ($2 \mu L$; 5×10^{-5} M in nitromethane) on the f.a.b. probe containing $5 \mu L$ of Cs Pic solution (5×10^{-2} M in PEG 200). Reference peak: $m/z = 151$; observed peak: $m/z = 1285$.

The curves shown in Figure 5 were obtained by recording the mass spectra after successive additions of $3 \mu L$ alkali picrate solution (B) on the f.a.b. probe containing $1 \mu L$ of CGD4Ac solution (5×10^{-4} M in nitromethane) and $4 \mu L$ of PEG 200 matrix.

- $B_{Cs} = B_{Rb} = B_K = 5 \times 10^{-5}$ M in nitromethane. Reference peak: $m/z = 239$. Observed peaks: $m/z = 1285$ (Cs$^+$); $m/z = 1237$ (Rb$^+$); $m/z = 1191$ (K$^+$).

In order to obtain the CGD4Ac–CsPic complexation curves (Figure 6) we recorded the mass spectra after successive additions of $x \mu L$ of cesium picrate solution in nitromethane (C) on the f.a.b. probe containing $1 \mu L$ of CGD4Ac solution (5×10^{-4} M in nitromethane) and $5 \mu L$ of PEG 200 matrix:

- molar ratio 0 to 20: $C_1 = 5 \times 10^{-4}$ M; $x = 0 \mu L$, $2 \mu L$ (10 times)
- molar ratio 0 to 30: $C_2 = 5 \times 10^{-4}$ M; $x = 0 \mu L$, $3 \mu L$ (10 times)
- molar ratio 0 to 100: $C_3 = 5 \times 10^{-4}$ M; $x = 0, 1, 1, 1, 2, 2, 3 \mu L$.
- molar ratio 0 to 420: $C_4 = 5 \times 10^{-3}$ M; $x = 0 \mu L$, $3 \mu L$ (14 times).

The reference and observed peaks were always at $m/z = 239$ and $m/z = 1285$ respectively. The complexation curves of 18C6 and CGD4Ac with CsCl in PEG 200 were obtained by recording the mass spectra after successive additions of $x \mu L$ of CsCl solution (D) on the f.a.b. probe containing $1 \mu L$ of 18C6 or CGD4Ac solution (5×10^{-4} M in water) and $5 \mu L$ of PEG 200 matrix:

- 18C6: $D_1 = 5 \times 10^{-1}$ M in water; $x = 0, 2, 2, 2, 3, 3 \mu L$. Reference peak: $m/z = 327$; observed peak: $m/z = 397$.
- CGD4Ac: $D_2 = 5 \times 10^{-3}$ M in water; $x = 0, 1, 2, 3, 3, 3, 3, 3 \mu L$. Reference peak: $m/z = 239$; observed peak: $m/z = 1285$.

3. Results and Discussion

3.1. COMPLEXATION STUDIES

In order to get quantitative measurements, all the $(18C6-M)^+$ peak intensities were normalized to the $(2G + H)^+$ peak at $m/z = 185$ of the glycerol (G) matrix. We found a concentration range in which the $(18C6-M)^+$ ion peak intensity was directly proportional to the $[18C6-M^+]$ complex concentration. In the particular case of KCl, Figure 1 shows the linear variation observed for the $(18C6-K)^+$ ion intensity at $m/z = 303$ as a function of $[18C6-K^+]$ complex concentration. This curve was obtained by adding a known amount of ligand to a solutoin of KCl $(4 \times 10^{-3} \text{ mol L}^{-1})$ in glycerol. Due to the large excess of K^+ compared to the ligand concentration, we can consider that all the 18C6 molecules are complexed. For concentrations of complex below 10^{-4} mol L^{-1}, the abundance of the $(18C6-K)^+$ ion increases linearly.

The complexation curves of 18C6 with alkali chlorides in glycerol (NaCl, KCl, RbCl and CsCl) have been obtained by measuring the peak intensity of the corresponding $(18C6-M)^+$ ion with increasing amounts of salt. In the following experiments the ligand concentration was 10^{-4} M in glycerol and the mass spectra were recorded after each addition of the alkali chloride in aqueous solution. Figure 2a shows the experimental complexation curve of 18C6 with KCl.

As mentioned previously the peak intensity of the $(18C6-K)^+$ ion is proportional to the complex concentration. We can then convert the peak intensity to complex concentration: after an infinite addition of salt all the ligand is complexed, and thus the complex peak intensity $I_{\text{asymptotic}}$ is proportional to the complex final concentration $[18C6-M^+]_{\text{final}}$ which is equal to the initial ligand concentration L_0, as shown in Equation (1):

$$I_{\text{asymptotic}} = P [18C6-M^+]_{\text{final}} = PL_0 \tag{1}$$

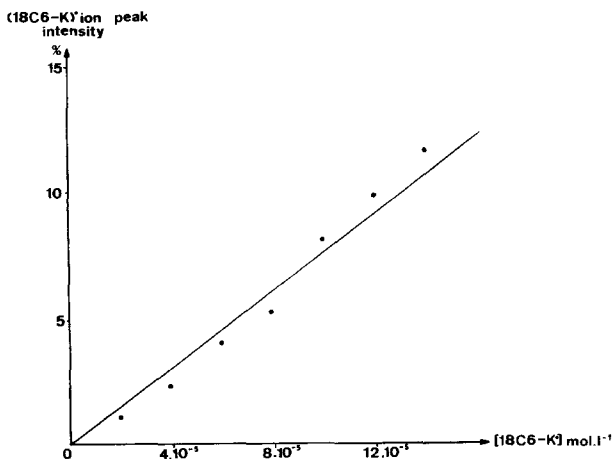where P is the proportionality factor.

Fig. 1. $(18C6-K)^+$ ion intensity as a function of the $[18C6-K^+]$ complex concentration. The intensities are normalized to the $(2G + H)^+$ ion peak at $m/z = 185$ of the glycerol (G) matrix which is set at 100%.
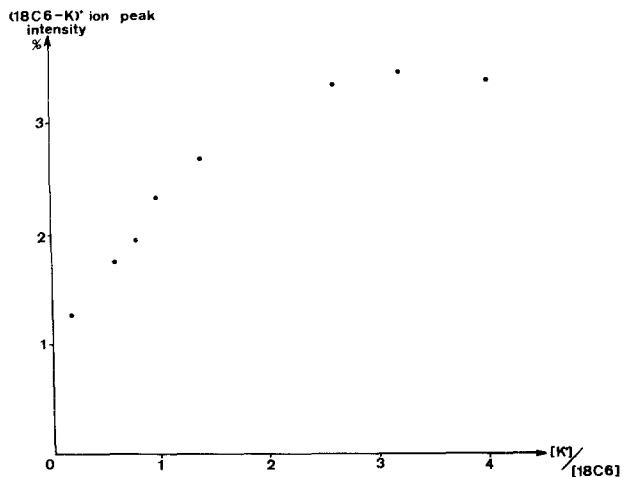
Fig. 2a. $(18C6–K)^+$ ion intensity as a function of the $[K^+]/[18C6]$ molar ratio. The intensities are normalized to the $(2G + H)^+$ ion peak at $m/z = 185$ which is set at 100%.
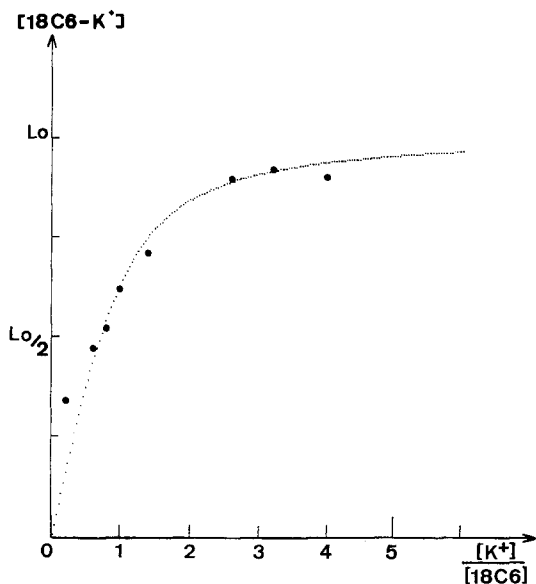
Fig. 2b. Computer simulated curves for the equilibrium $18C6 + K^+ \rightleftharpoons 18C6–K^+$. The calculated $[18C6–K^+]$ complex concentrations are plotted versus the $[K^+]/[18C6]$ molar ratio.

According to Equation (1), the complex peak intensities I_n measured after the nth addition of alkali salt can be expressed by Equation (2):

$$I_n = P\,[18C6–M^+]_n = PC_n \tag{2}$$

From Equations (1) and (2) the complex concentration C_n can be calculated from Equation (3):

$$C_n = \frac{I_n}{I_{\text{asymptotic}}} \times L_0 \tag{3}$$

However, $I_{\text{asymptotic}}$ cannot be obtained experimentally, in particular with weak complexes. Nevertheless, the complex concentration (C_n) can be calculated by iteration on the $I_{\text{asymptotic}}$ value.

For a metal ion M^+ in equilibrium with a crown ether 18C6 in solution (Equation (4)),

$$18C6 + M^+ \rightleftharpoons 18C6-M^+ \tag{4}$$

the corresponding stability constant K is given by Equation (5):

$$K = \frac{C_n}{(L_0 - C_n)(M_n - C_n)} \tag{5}$$

in which C_n and M_n are respectively the complex and cation concentrations after the nth addition of alkali salt, L_0 being the initial ligand concentration.

From Equation (5) we can obtain Equation (6) which directly relates the complex concentration C_n to the stability constant K:

$$C_n = \frac{KL_0 + KM_n + 1 - \sqrt{(KL_0 + KM_n + 1)^2 - 4K^2 L_0 M_n}}{2K} \tag{6}$$

The stability constant K is given by a curve fitting program [6] which calculates the best fit between the theoretical and experimental concentrations obtained from Equations (2) and (6), respectively, by successive iterations on the $I_{\text{asymptotic}}$ and K values (Figure 2b).

In the case of 18C6 in glycerol at 25°C the following stability constants were calculated:

$$\text{Na}^+: \log K = 2.32 \qquad \text{K}^+: \log K = 4.65$$
$$\text{Rb}^+: \log K = 3.79 \qquad \text{Cs}^+: \log K = 2.63$$

All these values are reported in Table I together with stability constants already published [5] concerning 18C6–M^+ complexes in different solvents.

We observe that there is a good agreement between the stability constants measured in glycerol or in 70% methanol, solvents which have similar dielectric constants.

Table I.   log K values[a] for 18C6–cation interaction in solution.

	H_2O	MeOH	70% MeOH	Glycerol
ε[b]	78.54	32.63	46.40[c]	42.50
Na$^+$	0.3–1.8	4.32–4.46	2.76	2.32
K$^+$	2.03–2.15	5.93–6.20	4.33	4.65
Rb$^+$	1.56	5.32–5.73	3.46	3.79
Cs$^+$	0.80–0.99	4.49–4.79	2.84	2.63

[a] Measured by calorimetric experiments (in 70% MeOH) or f.a.b.m.s. (glycerol). [b] Dielectric constant at 25°C. [c] Calculated (assuming a simple addition law for dielectric constants).

3.2. COMPLEXATION STUDIES OF CGD4Ac

Cyclogentiotetraose peracetate (CGD4Ac, **2**) has been synthesized [7] by internal cyclisation of bifunctionalized linear gentiotetraose.

Qualitative complexation studies between alkali cations and CGD4Ac have already been investigated by f.a.b.m.s. [8] The relative intensities of the (CGD4Ac–M)$^+$ ions showed the following affinities of CGD4Ac for the alkali cations:

$$Cs^+ > Rb^+ > K^+ > Na^+$$
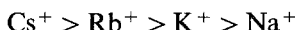
In this report we present quantitative studies of CGD4Ac complexation with Cs^+, Rb^+ and K^+ picrates in polyethylene glycol 200 (PEG 200). The (CGD4Ac–M)$^+$ complex peak intensities are normalized to the matrix peaks at $m/z = 151$ or $m/z = 239$.

We recorded the mass spectra after successive additions of ligand on a PEG 200 matrix containing cesium picrate in large excess. Under these conditions we can assume that all the ligand is complexed. As shown in Figure 3, for concentrations below 10^{-4} M, the (CGD4Ac–M)$^+$ peak intensity increases linearly with the complex concentration [CGD4Ac–M$^+$].

In order to compare the affinity of CGD4Ac with the following alkali cations, successive addition of K^+, Rb^+ or Cs^+ picrate solutions in nitromethane have been made to a PEG 200 matrix which contained a constant ligand concentration of 10^{-4} M. Figure 4 shows the linear variation of the (CGD4Ac–M)$^+$ peak intensity when the molar ratio [M$^+$]/[CGD4Ac] varies from 0 to 3. The slopes of the corresponding curves increase from K^+, Rb^+ to Cs^+ which confirms the greater affinity of the ligand towards cesium, in agreement with results already obtained either by f.a.b.m.s., or by proton and alkali cation NMR spectroscopy [9].

In the case of cesium picrate we obtained the complexation curves reported in Figures 5a and 5b and the equilibrium constants were calculated according to the method previously described for 18C6.

Several experiments for different molar ratios have been performed in order to estimate the accuracy of the method as shown in Figure 5b and Figure 6; the corresponding results are reported in Table II which indicate an average value of
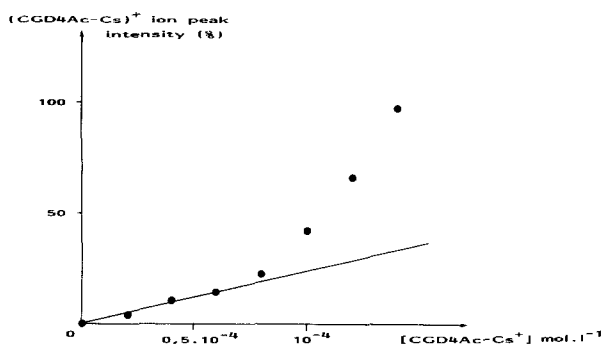

Fig. 3. (CGD4Ac–Cs)$^+$ ion peak intensity as a function of the [CGD4Ac–Cs$^+$] complex concentration. The intensities are normalized to the PEG 200 matrix peak at $m/z = 151$ which is set at 100%.
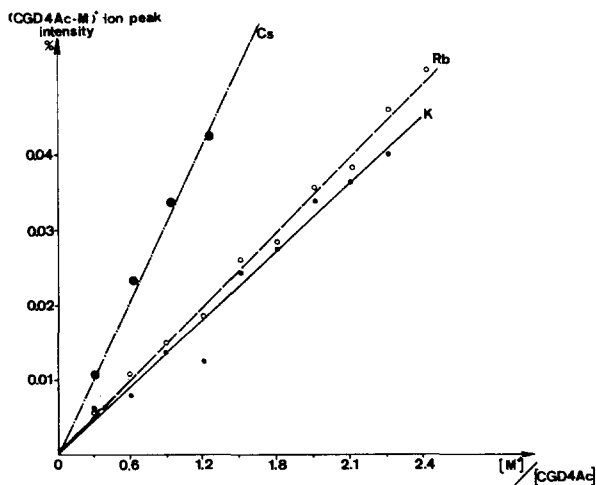
Fig. 4. (CGD4Ac–Ms)$^+$ ion peak intensity as a function of the [M$^+$]/[CGD4Ac] molar ratio. The intensities are normalized to the PEG 200 matrix peak at $m/z = 239$ which is set at 100%.

$\log K = 2.3 \pm 0.1$ for the equilibrium constant. We were not able to obtain the complexation curves of CGD4Ac in the case of K$^+$ or Rb$^+$ picrates because of their low solubility in nitromethane.

3.3. COMPARATIVE COMPLEXING PROPERTIES OF 18C6 AND CGD4Ac

In order to compare the respective affinity of 18C6 and CGD4Ac for cesium, the experiments have been done with cesium chloride in PEG 200 matrix for both ligands.
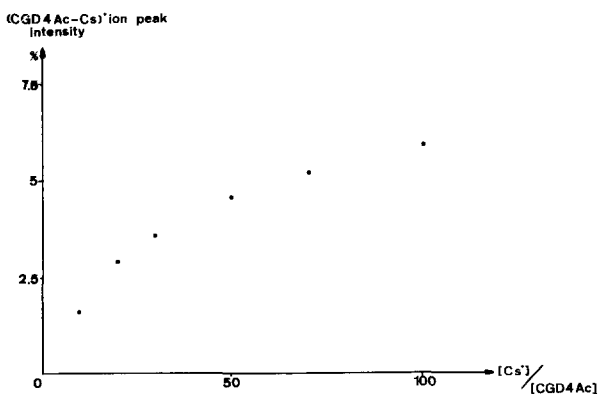

Fig. 5a. (CGD4Ac–Cs)$^+$ ion peak intensity as a function of the [Cs$^+$]/[CGD4Ac] molar ratio. The intensities are normalized to the PEG 200 matrix peak at $m/z = 239$ which is set at 100%.
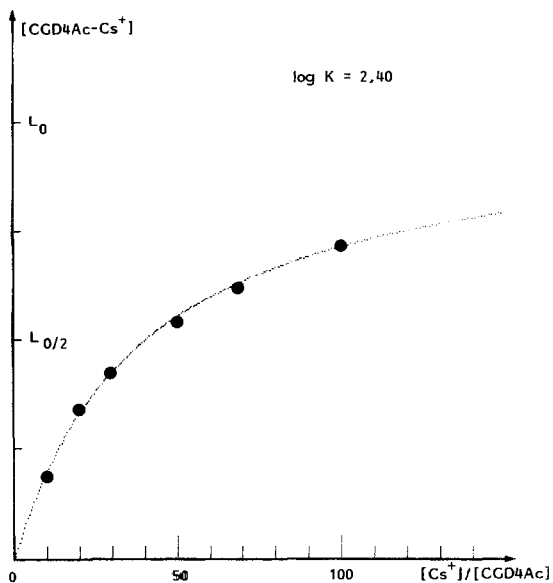
Fig. 5b. Computer simulated curve for the equilibrium $CGD4Ac + Cs^+ \rightleftharpoons CGD4Ac-Cs^+$. The calculated $[CGD4Ac-Cs^+]$ complex concentrations are plotted versus $[Cs^+]/[CGD4Ac]$ molar ratio (0–100).
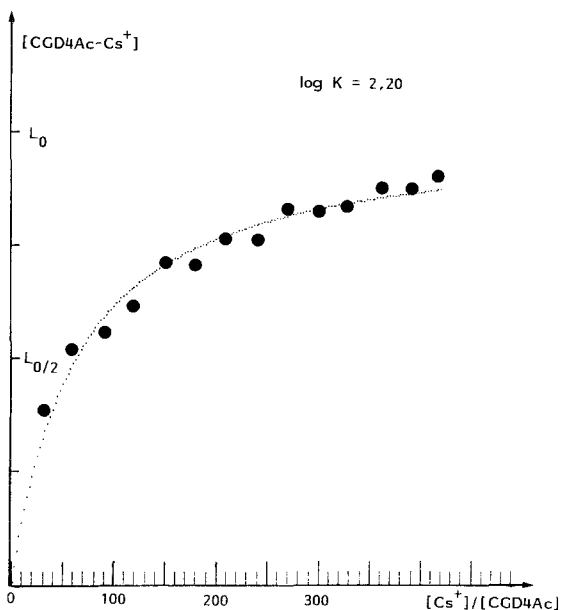

Fig. 6. Computer simulated complexation curve of CGD4Ac with Cs^+ at a 0–420 molar ratio range.

Table II.   log K values for CGD4Ac interaction with CsPic in PEG 200 matrix.

molar ratio $[Cs^+]/[CGD4Ac]$	log K
0–20	2.28
0–30	2.28
0–100	2.40
0–420	2.20

Table III.   log K values for 18C6 and CGD4Ac interaction with CsCl in PEG 200 matrix.

	18C6	CGD4Ac
log K	0.65 (2.63)[a]	2.45 (2.29)[b]

[a] log K value obtained with CsCl in glycerol matrix. [b] log K value previously obtained with CsPic in PEG 200 matrix.

The log K values obtained with the method previously described are reported in Table III.

As indicated by the log K values:

- the CGD4Ac–Cs^+ complex (log $K = 2.45$) is more stable than the 18C6–Cs^+ complex (log $K = 0.65$) in the PEG 200 matrix;
- the effect of the anion is weak and does not appear to play an important role in the complexation of CGD4Ac with the cesium salt in the PEG 200 matrix;
- the matrix properties have an important influence on the complexation reactions, in the case of 18C6 (log $K = 2.63$ and 0.65 in glycerol and PEG 200, respectively).

4. Conclusion

Complex formation between metal cations and macrocyclic ligands have been studied by fast atom bombardment mass spectrometry in glycerol and polyethylene glycol matrices. We describe a method to obtain complexation curves and to calculate stability constants for alkali cation-macrocyclic ligand interactions.

These results indicate the ability of the f.a.b.m.s. technique to investigate quantitative studies of cation-ligand complex formation for wide ranges of cations and many different types of ligands.

Acknowledgement

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