

The complexation of flavone derivatives with alkali and alkaline earth metal cations studied by spectroscopic methods

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A new kind of fluoroionophore, typified by flavone quasi-crown ether [4'-(dimethylamino)-3,6-(tetraethylene glycol)flavone] and flavone lariat-crown ether, were synthesized and characterized. The photophysical changes upon complexation with alkali and alkaline earth metal cations are explained in terms of the enhancement of a cation-induced intramolecular charge transfer process, which results from the interaction between the bound cation and the carbonyl group of the flavone. The size and shape of the quasi-crown or quasi-cryptand and the charge density of the bound cation control the magnitude of the photophysical changes. Based on the determination of stability constants between different cations and the macrocyclic ligand, our results indicate that the selectivity of flavone quasi-crown ether and flavone lariat-crown ether is better for the Ca^{2+} cation.

Fluorescence chemosensors is a new research field that has grown very rapidly.^{1–4} Obviously, it is closely related to the development of supramolecular chemistry and extensive studies on the mechanism of fluorescence emission.^{5,6} Structurally, fluorescence chemosensors consist of three moieties:⁷ a receptor, which is responsible for selectively binding a foreign species; a linker, which is a “bridge” used as a trigger between receptor and fluorophore; and a fluorophore, whose photophysical properties are sensitive to the binding between receptor and metal cations. Crown ethers, spherands and cryptands have served as template structures for cationic receptors, especially for the recognition of alkali and alkaline earth cations.^{8–10} The elegance of this work includes the designed architecture of the receptors, with consideration of the size and shape of the receptor site in comparison with the guest, which are used to rationalize observed binding data and to design new systems with unique properties.¹¹ The linker is a very important moiety in fluorescence chemosensor design, keeping in mind that the strongest perturbation of the photophysical properties of the fluorophore by the bound cation is sought. In many cases, the receptor is linked to the fluorophore *via* a linker that belongs to both the fluorophore and receptor. Recently, there has emerged a few fluorescence chemosensors in which the linker is not connected directly to the receptor; instead, it is part of the fluorophore and, by participating in the complexation, it acts as a trigger. For example, Valeur *et al.*⁶ reported the direct interaction between the bound cation and the carbonyl group of coumarin derivatives with lariat-crown ether. In this case the effect of bound cation on the carbonyl group is similar to the observation of a solvato-kinetic effect of coumarin derivatives in solvents with different polarity. In this paper, we report the synthesis and the photophysical properties of a new class of fluorescence

chemosensor—flavone quasi-crown ether (FQC) and flavone lariat-crown ether (FLC). The designed structure contains both an electron-donating amino group and an electron-withdrawing carbonyl group in the flavone moiety, so that it can undergo intramolecular charge transfer upon excitation by light. We also studied the photophysical changes upon complexation with alkali or alkaline earth metal cations.

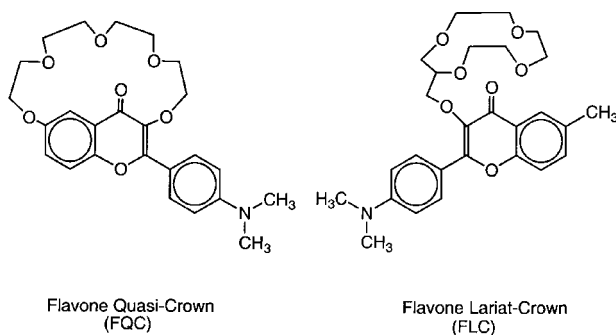
Experimental

Synthesis of FQC and FLC

4'-Dimethylamino-2,5-dihydroxychalcone. A mixture of 2,5-dihydroacetophenone (3.04 g, 20 mmol, synthesized according to ref. 12), *p*-dimethylaminobenzaldehyde (4.47 g, 30 mmol) and KOH (30 g) in methanol (50 mL) and water (25 mL) solution was stirred at room temperature for 24 h. After mixing with 32 g acetic acid and 300 mL water, the obtained clear solution turned a fresh red and a brown solid precipitated. The brown solid was recrystallized from 95% ethanol, giving 5.6 g of a brown powder, mp: 206–208 °C. ¹HNMR (DMSO-*d*₆) δ: 3.0 (s, 6H), 6.7–7.8 (m, 9H), 9.2 (s, 1H), 12.4 (s, 1H). MS (*m/z*): 283, 147, 134, 121, 103, 77, 53.

4'-Dimethylamino-2,7-dihydroxyflavone. Hydrogen peroxide (30%, 20 mL) was added dropwise to a DMSO (50 mL) solution of 4'-dimethylamino-2,5-dihydroxychalcone (5 g, 17.6 mmol) and KOH (20%, 25 mL) at 0 °C. After stirring for 4 h at room temperature, a blackish green solution was formed. After mixing the solution with glacial acetic acid and 300 mL water a solid product precipitated. After filtration, washing and recrystallization from 95% ethanol, a yellow powder was obtained, mp: 280–282 °C. ¹HNMR (CD₃COCD₃) δ: 3.05 (s, 6H), 3.4 (m, 2H), 6.8 (d, 2H), 7.2–7.6 (m, 3H), 8.2 (d, 2H). MS (*m/z*): 297, 268, 240, 149, 105, 89, 77, 52, 43.

1,11-Dibromo-3,6,9-trioxaundecane. A solution of tetraethylene glycol (17.3 mL) dissolved in dry ether (30 mL) was stirred under a dry atmosphere and cooled down to 0 °C. Into the solution, 8.5 mL of phosphorous tribromide was added dropwise, while keeping the temperature of the solution below 5 °C. After completion of the addition, the solution was allowed to warm up to room temperature and stirred for 4 h. The reaction mixture was then poured into a crushed ice bath, the separated organic layer was washed with a 10% sodium carbonate in water solution. The product was dried over



anhydrous potassium carbonate and the solvents evaporated to yield a yellow liquid. $^1\text{H NMR}(\text{CDCl}_3)$ δ : 3.4 (t, 4H), 3.63 (s, 8H), 3.7–3.8 (t, 4H). IR: 2868 (ν_{CH_2}), 1180 ($\nu_{\text{CH}_2\text{-O-CH}_2}$) cm^{-1} .

4'-(Dimethylamino)-2,7-(3,6,9-trioxaundecane-1,11-dioxy)-flavone (FQC). Anhydrous sodium carbonate (2 g) and 200 mL of DMF were placed in a dry three-neck round-bottom flask with two capillary dropping funnels and a reflux condenser. 1,11-Dibromo-3,6,9-trioxaundecane (2.8 g, 8.8 mmol) was dissolved in 80 mL of DMF in one funnel. 4'-Dimethylamino-2,7-dihydroxyflavone (1.3 g, 8.8 mmol), dissolved in 80 mL of DMF, was stored in the other one. The two solutions were added dropwise into the flask at the same rate at 100 °C, then the mixture was refluxed overnight. After completion of the reaction, the color of the mixture was a blackish green with fluorescence. The remaining DMF was removed by distillation. The remaining mixture was diluted with water and extracted from chloroform (3 \times 50 mL). A strongly fluorescent component could be separated from the concentrated extracted solution. Yellow crystals were obtained after recrystallization, mp: 280–282 °C, $^1\text{H NMR}(\text{CDCl}_3)$ δ : 3.1 (s, 6H), 3.1–3.2 (t, 2H), 3.35 (t, 2H), 3.45 (t, 2H), 3.7 (t, 2H), 3.8 (t, 2H), 4.2 (t, 2H), 4.5 (t, 2H), 6.8 (d, 2H), 7.2 (d, 1H), 7.4 (d, 1H), 7.9 (d, 1H), 8.1 (d, 2H). MS (m/z): 455, 412, 323, 280, 207, 162, 148, 132, 91, 77, 45.

3-Hydroxy-6-methyl-4'-N,N-dimethylaminoflavone (A). Hydrogen peroxide (30%, 10 mL) was added dropwise to a methanol (40 mL) solution of 4'-N,N-dimethylamino-2-hydroxy-5-methylchalcone (2 g, synthesized according to ref. 13) and 20% NaOH solution (10 mL) at 0–5 °C over 30 min. After being stirred for 24 h at room temperature, the bright yellow solution was neutralized with acetic acid. The yellow precipitate was filtered and washed with water. After drying the product was recrystallized from xylene to give 1.5 g (yield \approx 71%) of A as orange crystals, mp: 226–228 °C. MS (m/z): 295.

Flavone lariat-crown ether (FLC). To a vigorously stirred dry DMF (15 mL) solution of A (0.59 g), *tert*-butoxide potassium (0.23 g) and chloromethyl-12-crown-4 (0.49 g) were added at room temperature. The mixture was heated to 80–90 °C and maintained for 70 h. At the end of this time the mixture was cooled down, filtered and concentrated. The concentrated solution was poured into water (50 mL) and extracted with ethyl acetate (3 \times 50 mL). The organic layer was dried over sodium sulfate and the solvent then evaporated under vacuum. The crude product was purified by column chromatography and recrystallized from ethyl acetate–petroleum. Pure FLC (0.12 g) was obtained as yellow crystals, mp: 67–72 °C. $^1\text{H NMR}(\text{CDCl}_3)$ δ : 2.41 (s, 3H), 3.02 (s, 6H), 3.54–3.85 (m, 14H), 3.91–4.10 (m, 3H), 6.78–7.02 (d, 2H), 7.35–7.39 (m, 2H), 7.95 (s, 1H), 8.05–8.10 (d, 2H). MS (m/z): 483 (M^+). Elem. anal. calcd for $\text{C}_{27}\text{H}_{33}\text{NO}_7$: C, 67.06; H, 6.88; N, 2.90; found: C, 67.21; H, 7.11; N, 2.67.

Determination of the stability constants⁹

The stability constants can be obtained from the variation of either the absorption or fluorescence intensity at the appropriate wavelengths according to the following equations:

$$A_0/(A_0 - A) = \varepsilon_L/(\varepsilon_L + \varepsilon_{\text{ML}}) \times (1/K_s[\text{M}] + 1)$$

or

$$I_F^0/(I_F^0 - I) = \varepsilon_L \Phi_L/(\varepsilon_L \Phi_L - \varepsilon_{\text{ML}} \Phi_{\text{ML}}) \times (1/K_s[\text{M}] + 1)$$

where ε_L and ε_{ML} are the molar extinction coefficients of ligand and complex, respectively, and Φ_L and Φ_{ML} are the fluorescence quantum yields of ligand and complex, respectively. I_F^0 and A_0 are the absorption and fluorescence intensity of free

ligand, while I_F and A are the absorption and fluorescence intensity of the complex in solution at a given wavelength. The values of $A_0/(A_0 - A)$ or $I_F^0/(I_F^0 - I)$ are plotted against $[\text{M}]^{-1}$ and the stability constants K_s are then given by the ratio of intercept to slope.

It should be remembered that the stability constants obtained in the ground state are different from those of the excited state. Comparison of the two constants may provide us with some additional information.

Instrumentation and materials

The proton NMR spectra were recorded at 300 MHz on a Varian Germana-300. IR spectra were obtained on a Perkin-Elmer 938G spectrophotometer using KBr discs. Mass spectra were recorded on a Finnigan 4021C spectrometer. Absorption and fluorescence spectra were recorded on a Hitachi 330 UV-Vis spectrophotometer and MPF-4 fluorescence spectrophotometer, respectively.

Acetonitrile, purchased from Aldrich Chemical Inc. (spectroscopic grade), was used as solvent for the determination of absorption and fluorescence spectra without further purification, but it was checked by UV-Vis and fluorescence spectrophotometry prior to use. There was no absorption before 210 nm and no fluorescence of impurities was observed. Alkali metal and alkaline earth metal perchlorates, purchased from Beijing Chemical Reagent Inc., were of the highest quality available and vacuum dried over P_2O_5 prior to use.

Results and discussion

We previously reported the spectroscopic and photophysical properties of 4,4-dimethylaminoflavone.¹⁴ Its emission properties are strongly influenced by environmental stimuli such as the polarity and viscosity of the medium and the temperature.^{15,16} Flavone is a fluorophore in which the 4'-dimethylamino is an electron-donating group and the carbonyl group is an electron-acceptor, hence it is a donor–acceptor conjugated π -electron system, which can undergo intramolecular charge transfer upon excitation. It can thus be anticipated that the introduced cation in close proximity with the donor or acceptor moiety will change the photophysical properties of the fluorophore because the bound cation would affect the efficiency of intramolecular charge transfer.¹⁷ Roshal *et al.*¹⁸ have studied some flavone derivatives in which the amino electron-donating group was replaced by a monoaza-crown ether. Complexation reduced the conjugated system and resulted in a blue shift of the absorption spectrum. Conversely, a cation interacting with the carbonyl group enhances its electron-withdrawing character, thus a red shift of the absorption and fluorescence spectra of this compound is expected.

As exemplified by Fig. 1, the addition of alkali and alkaline earth metal perchlorates to solutions of FQC or FLC induces large red shifts in the absorption spectra of these crowns. An increase in the molar extinction coefficient also occurs. Furthermore, the alkaline earth cations lead to a stronger shift than the alkali metal cations. The emission spectra of free and bound FQC are very similar in shape and show a large red shift in the presence of cation perchlorates. A concomitant quenching of fluorescence intensity resulting from the enhancement of intramolecular charge transfer is also observed. For instance, the absorption spectrum of FQC is red-shifted about 36 nm upon complete complexation by Ca^{2+} . The spectral characteristics of free and fully complexed FQC and FLC are listed in Table 1. These data show that the order of the wavelength shift in the absorption and fluorescence spectra is $\text{Mg}^{2+} > \text{Ca}^{2+} > \text{Li}^{2+} > \text{Na}^+$ with both compounds. Moreover, the Stoke shift ($\Delta\nu$) seems also to exhibit a similar ordering. Obviously, the results are related to the charge density of the cations. The interactions between FQC

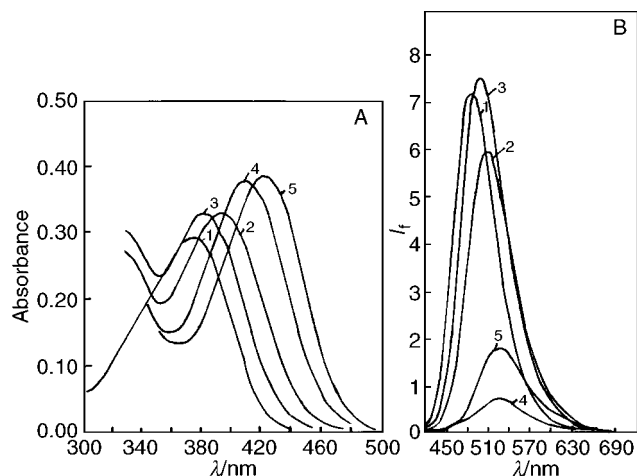
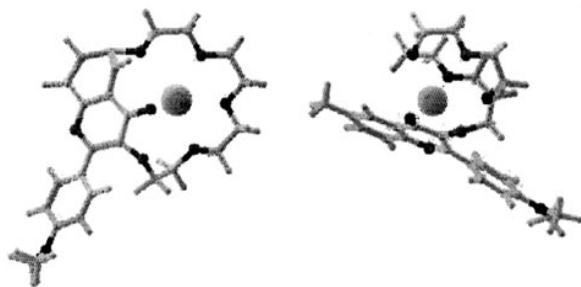


Fig. 1 Absorption and fluorescence spectra of FQC in acetonitrile and its complexes with different perchlorate salts. (A) Absorption and (B) fluorescence spectra: (curve 1) FQC, (curve 2) FQC-Li⁺, (curve 3) FQC-Na⁺, (curve 4) FQC-Mg²⁺, (curve 5) FQC-Ca²⁺.

or FLC and the cations were modelled and analyzed by PC Model 6.0 (Serena Software) software. However, in the calculation the solvent was not taken into account; thus the configurations obtained only provide an indication of what can happen. The configurations obtained from the calculations for FQC and FLC complexed with the four different cations are based on a 15-C-5 crown ether and a 15-C-5 cryptand, respectively (as shown in Scheme 1).

It can be clearly shown that the oxygen of the carbonyl group is also bound to the cation in FQC and FLC, while the oxygen at the 2 position in FQC does not bind with the cations. The involvement of the carbonyl group enhances the efficiency of intramolecular charge transfer, resulting in photophysical changes in the flavone fluorophore upon complexation with cations. These results are also supported by the change in the C=O absorption in the IR spectrum upon complexation with cations (see Table 2 below). In addition, from the experimental results it is seen that the monovalent ion



Scheme 1 Probable molecular configurations of FQC and FLC when complexed with different cations.

Na⁺ is difficult to complex with these crown compounds owing to its low charge density and big ionic diameter (0.19 nm). In Table 1, the data given for Na⁺ refer to partial complexation only and should be used with care when comparing the data.

The effect of gradual addition of different perchlorate salts on the absorption and fluorescence spectra of FQC and FLC in acetonitrile is shown in Fig. 2 for FLC. It is seen that for alkaline earth metal perchlorates, the absorption spectra show a well-defined isosbestic point in the whole range of concentration, whereas for lithium perchlorate the isosbestic point is only observed at high concentration due to its poor binding capability. From these spectra we are able to plot the absorption changes of the flavone derivatives against the concentration of alkali and alkaline earth metal perchlorates; an example is given in Fig. 3. The ratio of $A_0/(A_0 - A)$ can then be plotted *vs.* $[M]^{-1}$, as in Fig. 4, to give a set of quite good straight lines. As it is well known that the Benesi-Hildebrand equation is derived on the basis of a 1 : 1 stoichiometric ratio, the linear relationship obtained indicates that the stoichiometric ratio of the host-guest complexes studied in this work is 1 : 1. In addition, it is also worth noting that Na⁺ and K⁺ can cause a red shift of the electronic spectra and fluorescence quenching of FQC or FLC at low concentrations. But their binding with the ligands is difficult to observe at low concentrations. From Fig. 3 it can also be seen clearly that the binding ability of Na⁺ (and even of Li⁺ to a certain extent) to

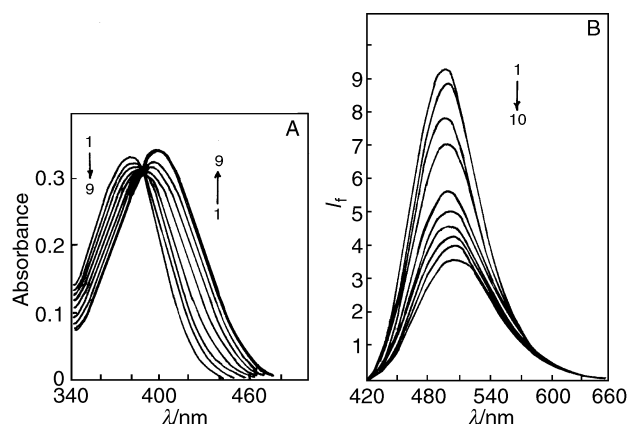


Fig. 2 Effect of the concentration of different metal perchlorates on the absorption and fluorescence spectra of FLC. (A) Effect of the addition of Mg²⁺ salt on the absorption spectrum of FLC (1×10^{-2} mM) in acetonitrile. Concentration of Mg²⁺: (curve 1) 0, (curve 2) 0.15, (curve 3) 0.5, (curve 4) 1.0, (curve 5) 2.0, (curve 6) 3.0, (curve 7) 5.0, (curve 8) 20.0, (curve 9) 30.0 mM. (B) Effect of the addition of Ca²⁺ salt on the fluorescence spectrum of FLC (1×10^{-2} mM) in acetonitrile. Concentration of Ca²⁺: (curve 1) 0, (curve 2) 0.15, (curve 3) 0.2, (curve 4) 0.35, (curve 5) 0.5, (curve 6) 0.74, (curve 7) 0.99, (curve 8) 1.23, (curve 9) 1.48, (curve 10) 1.73 mM.

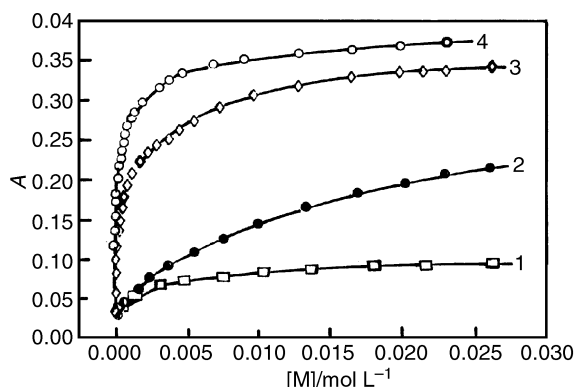
Table 1 The spectrum characteristics of free and complexed FQC and FLC

	$\epsilon/L \text{ mol}^{-1} \text{ cm}^{-1}$	$\lambda_{\text{max}}/\text{nm}$			$\Delta\nu_{\text{Stokes shift}}/10^3 \text{ cm}^{-1}$
		Absorption	Emission		
FQC	29 200	374	489		8.70
FQC-Li ⁺	32 300	394	510		8.62
FQC-Na ⁺ ^a	32 500	380	504		8.06
FQC-Mg ²⁺	32 800	423	528		9.52
FQC-Ca ²⁺	37 100	410	525		8.70
FLC	21 900	375	492		8.55
FLC-Li ⁺	21 400	386	506		8.33
FLC-Na ⁺	21 900	380	498		8.47
FLC-Mg ²⁺	23 500	404	520		8.62
FLC-Ca ²⁺	22 900	402	520		8.47

^a The Na⁺ complexation is discussed in more detail in the text.

Table 2 The stability constants for complexation between FQC or FLC and different metal cations

	Ligand	Li ⁺	Mg ²⁺	Ca ²⁺
Diameter/nm	0.17–0.22 ^a	0.136	0.132	0.198
Log <i>K</i> _{abs} (FQC)	—	1.51 ± 0.01	2.98 ± 0.02	3.19 ± 0.01
Log <i>K</i> _{em} (FQC)	—	1.68 ± 0.02	3.19 ± 0.02	3.34 ± 0.01
Log <i>K</i> _{abs} (FLC)	—	—	3.38 ± 0.02	5.52 ± 0.03
Log <i>K</i> _{em} (FLC)	—	3.02 ± 0.03	3.27 ± 0.04	5.48 ± 0.02
ν(C=O)/cm ⁻¹	1634	1625	1612	1616

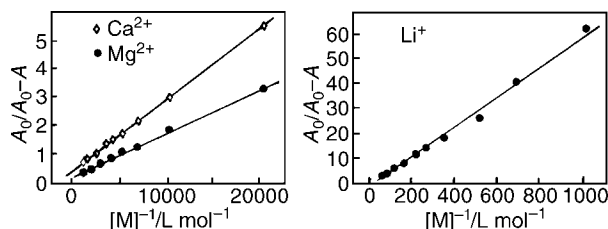
^a Cavity size of the crown ether (15-C-5).**Fig. 3** The change in absorption of FQC (1×10^{-2} mM) in acetonitrile vs. the concentration of alkali and alkaline earth metal perchlorates (curve 1) Na⁺, (curve 2) Li⁺, (curve 3) Mg²⁺, (curve 4) Ca²⁺. The measured wavelength is set at the absorptive peak wavelength of each compound.

the hosts used here is not strong enough. The changes in the host absorption are not evident when the host is complexed with these cations at low concentrations. Hence, only the Li⁺,²⁰ Mg²⁺ and Ca²⁺ complex stability constants can be obtained with certainty. These are listed in Table 2.

It is apparent that the stability constants of the divalent alkaline earth metal cations are far larger than those of the monovalent alkali metal cations. This demonstrates that the complex stability constants of FQC or FLC with metal cations are related closely to the charge density of the cation and the electrochemical properties of the receptor. For divalent alkaline earth metal cations, the stability constant of Mg²⁺ is smaller than Ca²⁺, consistent with the complexation of regular 15-C-5-crown ether with alkali and alkaline earth metal cations. In addition, the complex stability constants of FLC with cations are larger than those of FQC. This result is consistent with previous literature reports.¹⁹

Conclusion

In summary, we find that there is some analogy in structure between FQC studied in this work and the crown ethers derived from 2,7-dihydroxyacridan-9-one,⁷ but remarkable differences between them have also been observed. For instance, acridan-9-one crown ether cannot complex with alkali metal and alkaline earth metal cations but with Cu⁺, Ag⁺ and Fe²⁺. Conversely, FQC still has some hard base

**Fig. 4** Plots of $A_0/(A_0 - A)$ against the inverse of the metal ion concentration $[M]^{-1}$.

character and can bind with alkali and alkaline earth metal cations. Obviously, this is due to the chemical structure of FQC. Flavone quasi-crown ether and flavone lariat-crown ether are selective in cation recognition due to the photochemical properties of their flavone fluorophore, e.g., the appropriate intramolecular charge transfer character, the high fluorescence quantum yield, etc. Although their complex stability constants with Ca²⁺ are prominent, their suitability for the recognition of other cations is under further investigation.

Acknowledgements

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