The Synthesis of 1,4-Diaza-2,3;8,9-dibenzo-7,10-dioxacyclododecane-5,12-dione and Its Use in Calcium-Selective Carbon Paste Electrodes

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Abstract

A synthetic procedure has been developed for the preparation of 1,4-diaza-2,3;8,9-dibenzo-7,10-dioxacyclododecane-5,12-dione. A carbon paste electrode based on the new macrocyclic diamide was constructed for Ca^{2+} determination. The calibration graph was linear over a wide concentration range (1.3×10^{-6}–3.2×10^{-3} M) with a near Nernstian slope of 32 mV decade^{-1}. The limit of detection is 7.9×10^{-7} M (0.032 ppm). Selectivity coefficients were tabulated and the working pH range was determined. It has a fast response time of <10 s and can be used for at least 3 months without any considerable divergence in potentials. The electrode was successfully applied to the determination of Ca^{2+} ions in a pharmaceutical preparation.

Introduction

The design and synthesis of new functionalized macrocyclic ligands for specific analytical applications is a subject of continuous recent interest [1–3]. The in-built configuration rigidity induced by N-substituted amides present in the periphery of benzomacrocycles invokes preorganization leading to ionophoric selectivity [4–8]. Thus, we have recently reported the successful use of some different size benzosubstituted macrocyclic diamides as ion carriers in construction of PVC-based selective electrodes for Cu(II) [9], Zn(II) [10], Hg(II) [11] and Sr(II) [12].

During the past two decades, considerable attention has been focused on electrochemical determinations using chemically modified carbon paste electrodes, with the preconcentrating agent mixed into a carbon paste matrix in order to react with and bind selectively the target solute [13–18]. The resulting electroresponses are very stable and the electrodes can be easily renewed by removing an outer layer of the paste and re-smoothing the surface. Yet, the applications of modified paste electrodes are mainly defined in the field of voltammetric determination and very few have been used in potentiometry. Some potentiometric modified carbon paste electrodes are reported for the determination of chloride [19], chromium (VI) [20], perchlorate and fluoroborate [21], Cu(II) [22] and s-captopril [23].

In this paper we report the first synthesis of 1,4-diaza-2,3;8,9-dibenzo-7,10-dioxacyclododecane-5,12-dione (**L**) and its use as a suitable electroactive material for construc-

tion of a potentiometric carbon paste modified electrode for Ca^{2+} ion. The advantages of using a carbon paste electrode involved ease of construction, increased portability, robustness and economy.
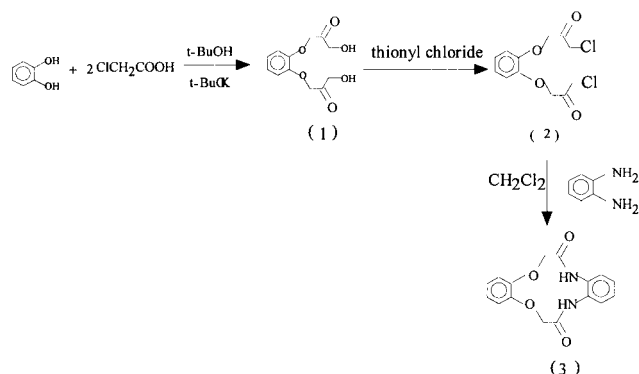

(L)
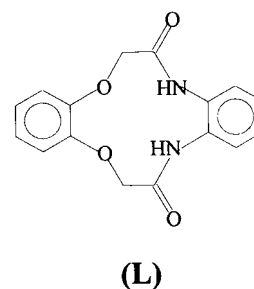
Experimental

Reagents

Doubly distilled deionized water was used throughout. Reagent grade acetophenone (AP), tetrahydrofuran (THF), paraffin oil and graphite powder (<0.1 mm) were purchased from Merck chemical company and used as received. The nitrate salts of all cations used (Merck) were of the highest purity available and used without any further purification except for vacuum drying over P_2O_5. All other chemicals were purchased from Fluka or Merck chemical companies and used as received.

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Synthesis of ionophore

1,4-Diaza-2,3;8,9-dibenzo-7,10-doxacyclododecane-5,12-dione (**L**, **3**) was synthesized in three steps as follows.

Phenylenedioxydiaceltic acid (**1**)

Catechol (11 g 0.1 mole) and potassium t-butoxide (61.7 g, 0.55 mole) were refluxed with stirring for 30 min in 350 mL t-butanol. A solution of chloroacetic acid (18.9 g, 0.2 mole) in 150 mL t-butanol was then added over a 45 min period and refluxing and stirring were continued overnight. The mixture was poured into ice/water, t-butanol was removed in vacuum, and the resulting solution was extracted with chloroform. The aqueous phase was acidified with concentrated hydrochloric acid and the resulting solid was filtered out. Crystallization of the residue from hot water afforded a white solid of (**1**) (20.5 g, 91% yield, m.p. 178–179 °C). IR (KBr, cm^{-1}): 662 (w), 753 (m), 781 (w), 882 (w), 858 (w), 1062 (m), 1139(s), 1029 (s), 1232 (s), 1402 (w), 1433 (m), 1508 (s), 1598 (w), 1740 (s), 2535 (w), 2631 (w), 2850 (w). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 465 (s, 4H), 6.88 (s, 411). MS m/z (%): 228 (M$^+$ + 2, 1.0), 227 (M$^+$ + 1, 7.5), 226 (M$^+$, 55.3), 208 (0.6), 167 (2.4), 151 (16.4), 135 (6.1), 123 (39.0), 121 (33.7), 109 (base peak), 77 (21.2).

2-Phenylenedioxydiacetyl chloride (**2**)

1,2-Phenylenedioxydiacetic acid (**1**) (5.65 g, 0.025 mole) was heated in 50 mL thionyl chloride for 4 h at 50–60 °C. The solvent was slowly evaporated at low temperature to give compound (**2**) as a white cream solid (5.4 g, 85% yield; m.p. = 48–49 °C). The structure of compound (**2**) was confirmed by ^{1}H NMR and IR spectroscopy.

1,4-Diaza-2,3;8,9-dibenzo-7,10-dioxacyclooociadecane-5,12-dione (**L**, **3**)

A solution of o-phenylenediame (0.22 g, 2 mmole) in dry CH$_2$Cl$_2$ (50 mL) was added quickly to a vigorously stirring solution of compound **2** (0.53 g, 2 mmole) dissolved in 50 mL CH$_2$Cl$_2$ at room temperature. The mixture was stirred for 20 min and then was washed with a bicarbonate solution (2 × 50 mL). The organic layer was dried over magnesium sulfate and the solvent was evaporated to give a solid (or oily) product. The crude product was purified by column chromatography over silica gel using petroleum ether/ethyl acetate as eluent. A pure white solid was obtained in 71% yield (0.043 g, m.p. = 213–214 °C). IR (KBr, cm^{-1}): 623 (w), 692 (w), 731 (m), 1027 (w), 1035 (w), 1105 (m), 1123 (w), 1188 (m), 1255 (m), 1290 (w), 1445 (m), 1490 (s), 1590 (m), 1668 (s), 2850 (w), 2913 (m), 3030 (w). 3301 (s). ^{1}H NMR (CD$_3$COCD$_3$) δ (ppm): 4.70 (s, 4H), 6.99 (dd, 2H, J_1 = 7.15 Hz, J_2 = 2.55 Hz), 7.23 (dt, 4H, J_1 = 5.65 Hz, J_2 = 2.00 Hz), 7.64 (dd, 2H, J_2 = 9.90 Hz, J_2 = 2.50 Hz), 9.27 (b, 2H). Mass m/z (%): 300 (M$^+$ + 2, 0.3), 299 (M$^+$ + 1. 2.6), 298 (M$^+$, 12.7), 176 (3.5), 175 (28.5), 148 (10.1), 147 (44.8), 137 (6.2) 134 (82.8), 133 (10.0), 132 (13.4), 121 (25.2), 120 (13.9), 119 (base peak) 118 (6.6). 106 (13.8), 105 (12.0), 92 (16.6), 91 (5.4), 77 (19.8). UV (CHCl$_3$), $\lambda(\epsilon_{max})$: 254.5 nm (2551). Chem. Anal., Calcd for C$_{16}$H$_{14}$N$_2$O$_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.51; H, 4.66; N, 9.42.

Electrode preparation

Modified electrodes were prepared by mixing 63 mg graphite powder and 17 mg of powdered ionophore **L** in an agate mortar. Then 36 mg AP were added and the mixture was mixed until a uniform paste was obtained. The paste was packed into electrode assemblies made of 1 mL polypropylene insulin syringes. Electrical contact was made by means of a copper wire passed through the syringe piston. The fresh surfaces were obtained by polishing the electrode on a clean paper until they showed a smooth and shiny appearance after every measurement.

Apparatus

All EMF measurements were carried out with a Metrohm ion analyzer model 632 pH/mV meter, equipped with a saturated Ag/AgCl reference electrode. The modified carbon paste electrode described above was used as the indicator electrode. Activities were calculated according to the Debye–Hückel procedure [24].

The NMR spectra were recorded using a Bruker Advance DPX-250 spectrometer in pure deuterated solvents with TMS as an internal standard. The IR spectra were obtained on an Impact 400D Nicolet FTIR spectrometer. The mass spectra were determined on a Shimadzu GC-MS-QP 1000 EX instrument at 70 or 20 eV. The UV-Vis spectra were recorded on a Philips PUB 700 spectrophotometer. The conductance measurements were carried out with a Metrohm 712 conductivity meter. A dip-type conductivity cell made of platinum black was used.

Results and discussion

Ligands for use as ionophores in a calcium ion-selective electrode should fulfill certain conditions including: (1) increased selectivity for Ca^{2+} over other metal ions, (2) rapid exchange kinetics of the resulting complex and (3) sufficient lipophilicity to prevent leaching of the ligand into the surrounding aqueous solution. The selectivity of ordinary crown ethers for alkaline earth metal ions is much lower than that for alkali cations [25, 26]. However, the introduction of an amide linkage in the polyether ring has been clearly
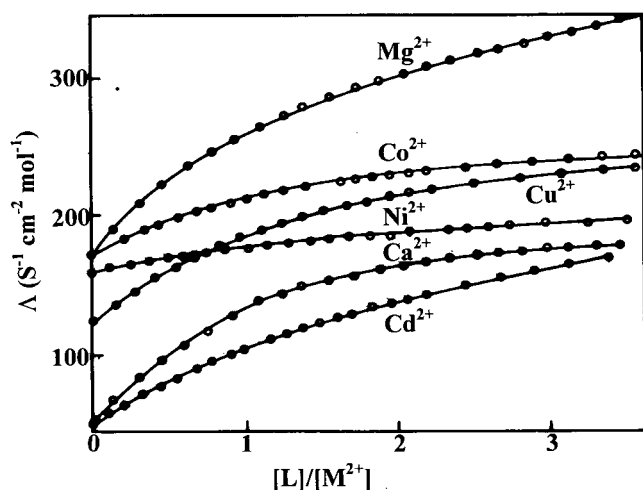
Figure 1. $\Lambda(S^{-1} \text{ cm}^{-2} \text{ mol}^{-1})$ vs. $[L]/[M^{n+}]$ in acetonitrile solution at 25 °C.

Table 1. Formation constants, K_f and molar conductance, Λ_{ML}, of 1:1 complexes between different cations and macrocyclic diamide **L** in acetonitrile solution at 25 °C

Cation	log K_f	Λ_{ML} (S^{-1} cm^{-2} mol^{-1})
Mg^{2+}	4.49 ± 0.03	373 ± 5
Ca^{2+}	4.80 ± 0.04	258 ± 4
Co^{2+}	4.11 ± 0.02	273 ± 2
Ni^{2+}	3.23 ± 0.15	311 ± 4
Cu^{2+}	4.26 ± 0.04	294 ± 6
Cd^{2+}	3.80 ± 0.05	329 ± 1

shown to modify the binding properties of the crown compounds in favor of alkaline earth with respect to alkali metal ions [27–30]. Moreover, amide substitution in the cavity of benzocrown ethers may not only contribute to their cation selectivity but also allow the macrocycles to have properties more closely resembling those of naturally occurring ionophores [31]. Thus, we have previously reported the successful use of some benzo-substituted macrocyclic diamides as suitable ionophores in the preparation of PVC-based ion-selective electrodes [9–12]. In this work the suitable cavity size of the macrocyclic diamide derivative **L** as well as its water insolubility led us to examine its ability as a potential ion carrier in carbon paste electrodes selective for Ca^{2+} ion.

In preliminary experiments, the complexation of **L** with a number of alkaline earth and transition metal ions was studied conductometrically in acetonitrile solution at 25.00 $\pm$ 0.03 °C [32–34], in order to obtain information about the stability and selectivity of the resulting complexes. The resulting molar conductance, Λ, vs. $[L]/[M^{2+}]$ mole plots are shown in Figure 1. As seen, in most cases, addition of the ligand to the cation solution caused a rather large and continuous increase in the molar conductance of solutions, indicating the higher mobility of the complexed cations compared to the solvated ones. The increased molar conductance of the metal nitrates in acetonitrile solution upon addition of the ligand can also be related to some extent to the dissociation of some ion-paired species usually present in acetonitrile, as a solvent of intermediate dielectric constant and relatively low solvating ability, as a result of the metal ion complexation with **L** [32]. The complex formation constant, K_f, and the molar conductance of the resulting 1:1 complexes, λ_{ML}, were evaluated by computer fitting of the molar conductance-mole ratio data to appropriate equations [32–34], and the results are summarized in Table 1. From the data given in Table 1, it is immediately obvious that **L** could act as a selective ionophore for Ca^{2+} ion in a carbon paste electrode.

The performance characteristics reported for a given ionophore vary depending on electrode composition and the nature of solutions (e.g., ionic strength, pH) to which the electrodes are exposed. In primary experiments it was found that, while the use of plain carbon paste electrodes resulted in no measurable response with respect to Ca^{2+} ions over a wide concentration range (i.e., from 10^{-7} to 10^{-1} M), the addition of ionophore **L** showed a near-Nernstian response of the modified CPE for the cation in the range of 1.0×10^{-6}–1.0×10^{-3} M. The optimum amount of ionophore **L** was investigated and found to be 15 wt-wt%.

Three different solvents, dioctyl phthalate, paraffin and acetophenone were tested for the preparation of modified carbon pastes. The results revealed that the CPE with acetophenone had the best calibration slope, the fastest response rates, the lowest detection limit and the widest linear range (Figure 2). A graphite: acetophenone: ionophore percent ratio of 55: 30: 15, obtained from optimization studies of the electrode (with a slope of 31.9 ± 0.4 mV per decade) over a wide concentration range of 1.3×10^{-6}–3.2×10^{-3} M was used. A sample calibration graph for the electrode is shown in Figure 3. The limit of detection of calcium, as determined from the intersection of the two extrapolated segments of the calibration graph, was 7.9×10^{-7} M.

The modified CPE prepared with the optimal ratios was found to have a very short response time for Ca^{2+} ion monitoring. The response time ($t_{90\%}$) measured was less than 10 s for a calcium ion concentration of 1.0×10^{-3} M or less. The potential variation of the electrodes was within ±0.3 mV day-to-day results. The lifetime of the modified carbon paste electrode was at least 3 months, during which it could be used without any measurable divergence.

The influence of pH on the response of the proposed electrode to 1.0×10^{-3} M Ca^{2+} concentration over a pH range from 2 to 10 (maintained by either nitric acid or tetraethyl-ammonium hydroxide solutions) was studied, and the results are shown in Figure 4. As seen, the CPE can be suitably used in the range of 3–9. However, the observed potential changes below and above this pH range may be due to protonation of the macrocyclic diamide and Ca^{2+} hydrolysis, respectively.

The extent of the interference of various cationic species on the proposed Ca^{2+} ion-selective sensor was studied by the mixed solution method [35, 36], from potential measurements of the solutions containing a fixed amount of Ca^{2+} ion ($a_{Ca} = 1.0 \times 10^{-4}$ M) and varying concentrations of the interfering ion (M^{n+}, a_M) according to:
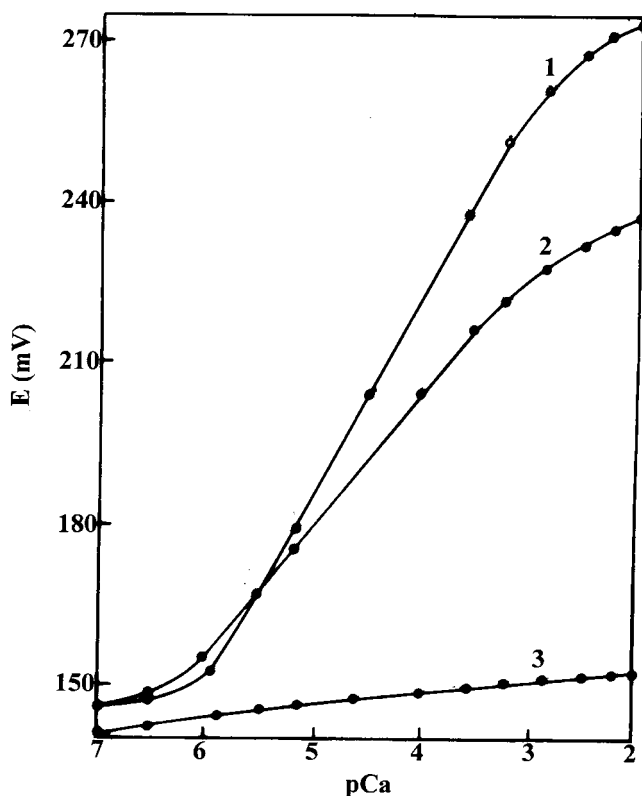
Figure 2. Effect of different solvents on the potential response of the modified carbon paste electrode: (1) acetophenone, (2) paraffin, (3) dioctylphthalate.
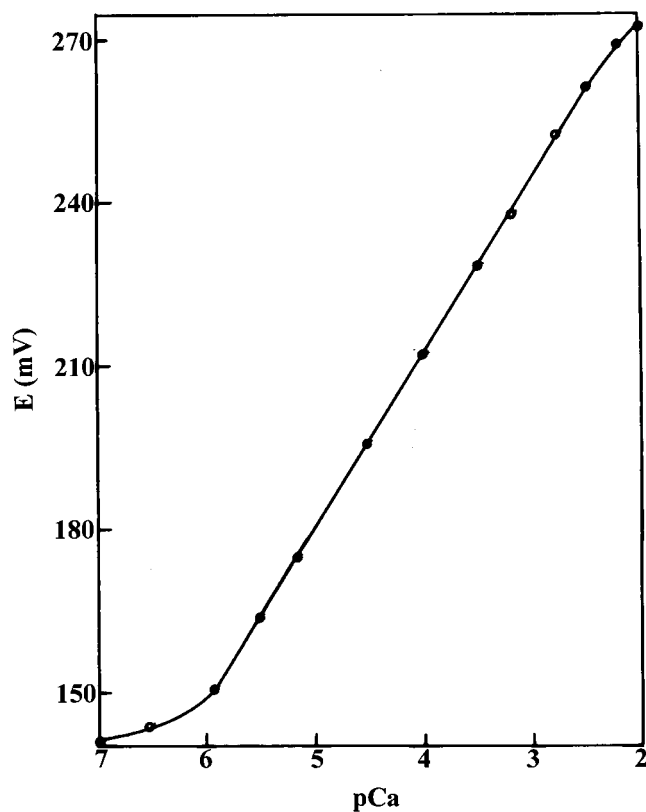

Figure 4. Effect of the pH of the test solution on the potential response of the Ca^{2+} ion-selective electrode. The test solution was 1.0×10^{-3} M Ca^{2+}.

Table 2. Selectivity coefficients of various interfering ions

M^{n+}	K_{Ca}^{Pot}	M^{n+}	K_{Ca}^{Pot}
Cr^{3+}	4.0×10^{-3}	Cd^{2+}	5.4×10^{-2}
Mg^{2+}	2.0×10^{-2}	Pd^{2+}	3.2×10^{-2}
Sr^{2+}	$<1.0 \times 10^{-4}$	Li^+	2.4×10^{-1}
Ba^{2+}	5.0×10^{-3}	K^+	3.5×10^{-1}
Ni^{2+}	1.6×10^{-3}		

Figure 3. Potential response of the proposed calcium-selective carbon paste electrode. Supporting electrolyte was 0.1 M tetraethylammonium perchlorate at pH 5.5.
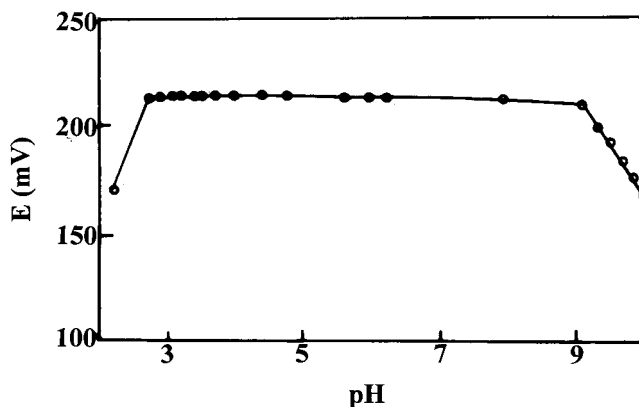
$$K_{Ca}^{Pot} a_M^{2/n} = a_{Ca}\{\exp[(E_2 - E_1)F/RT]\} - a_{Ca}, \quad (1)$$

where E_1 and E_2 are the electrode potentials for the solution of Ca^{2+} ion alone and for the solution containing interfering ions and calcium ions, respectively. According to Equation (1), the K_{Ca}^{Pot} value for different cations can be evaluated from the slope of the graph of $a_{Ca}\{\exp[(E_2 - E_1)F/RT]\}$ vs a_{Ca}. The resulting K_{Ca}^{Pot} values are summarized in Table 2. As can be seen, in the case of bivalent and trivalent interfering ions, the selectivity coefficients are in the order of 10^{-2} or smaller which seems to indicate that these metal ions have negligible disturbance of the functioning of the Ca^{2+} ion-selective CPE. It should be noted that in the case of univalent interfering ions, the selectivity coefficients seen to be somewhat larger. However, such larger K_{Ca}^{Pot} values arise from the term $a_M^{2/n}$ in Equation (1); the smaller the charge of the interfering ion, n, the larger the selectivity coefficient. Thus, despite their larger selectivity coefficients, the univalent ions tested would not disturb the functioning of the CPE significantly.

In order to investigate the practical utility of the proposed Ca^{2+}- selective CPE to real samples, it was applied to the determination of the calcium content of a pharmaceutical preparation, calcium gluconate, using the standard addition method. The calcium content obtained from three replicate measurements (9.65 ± 0.20 mg Ca per 1 mL drug) was found to be in satisfactory agreement with that obtained by atomic absorption spectrometry (9.50 ± 0.12 mg Ca per 1 mL drug).

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