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New carbazolo[1,2-*a*]carbazole derivative as ionophore for anion-selective electrodes: Remarkable recognition towards dicarboxylate anions

María Cuartero [a], Miriam Más-Montoya [b], M. Soledad García [a], David Curiel [b,*], Joaquín A. Ortuño [a,**]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Murcia E-30100, Spain
[b] Department of Organic Chemistry, Faculty of Chemistry, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Murcia E-30100, Spain

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ABSTRACT

A new carbazolo[1,2-*a*]carbazole derivative was synthesized by expanding the binding cavity to explore the possibility of hosting larger anions such as dicarboxylate anions. The compound was incorporated as an ionophore into a membrane for an anion-selective electrode. The response of the electrode was evaluated for oxalate, malonate, succinate, glutarate and adipate in terms of calibration characteristics (slope, limit of detection and linear range of the response), response time, repeatability, reproducibility and selectivity. Nernstian reproducible responses, with very good detection limits, fast responses and selectivity not previously observed, were found for all the dicarboxylates anions, and the results were especially good in the case of glutarate. In order to obtain additional structural information about the complex formed between the ionophore and the dicarboxylate anions, ^{1}H NMR and fluorescence studies were carried out. The observed potentiometric selectivity depends on the good correspondence between the size of the carbazolocarbazole cavity and the length of the dicarboxylate anion, as supported by the NMR and fluorescence studies.

1. Introduction

An ionophore has been defined as a lipophilic complexing agent incorporated into a membrane that reversibly binds ions [1]. Ionophores can be designed to gain certain selectivity in the response of an ion-selective electrode (ISE) [2]. The first progress of ionophore-based ISEs development was inspired by the work of Moore and Pressman about the uptake of potassium by the antibiotic valinomycin in 1964 [3]. This finding completely transformed ISEs research, representing the onset of the host–guest chemistry help on the development of new sensors [4]. Although the first works on cation and anion receptors date back to the same time [5,6], development of ionophore-based ISEs for cations progressed faster. The development of ISEs for anions with little tendency to enter the membrane is still a challenge. This is due to the difficulty involved in finding anion-selective ionophores that provide a potentiometric response, which is mainly determined by the selective complexation of the anion with the ionophore [7]. Such is the case of the oxalate anion and the set of dicarboxylate anions, malonate, succinate, glutarate and adipate.

Ionophores proposed for anion-selective electrodes are of three types: macromolecules with a Lewis acid-type metal center, non-covalent binding compounds (hydrogen-binding and ion-dipole) and covalent-binding compounds. The most used ionophores have been compounds of the first type and [8] although hydrogen-bonding compounds are increasingly used [9]. The selectivity of ionophores containing a Lewis acid metal is mainly controlled by the interaction of the metal center with the anion, although it can be modulated by the ligands present in the macromolecule. As regards hydrogen-binding receptors, most of them are a combination of NH and CH hydrogen-binding sites, which can be arranged in cyclic or acyclic architectures. This provides the receptor a degree of geometrical selectivity [10]. Ionophores recently described in the literature used in electrodes for halide [9], carbonate [11] and sulfate [12] anions belong to this latter type of receptor.

* Corresponding author. Tel: +34 868 888389; fax: +34 868 364149.
** Corresponding author. Tel.: +34 868 887414; fax: +34 868 887682.
E-mail addresses: davidcc@um.es (D. Curiel), jortuno@um.es (J.A. Ortuño).

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The first aim of the present work was to synthesize a new receptor by expanding the binding cavity of the carbazolocarbazole system in an attempt to increase the possibility of hosting larger anions such as dicarboxylates. The determination of some dicarboxylate anions is important because of many reasons. The oxalate anion is found in nature in various types of plants, such as the poisonous plant *Dieffenbachia* [13]. Its measurement in urine is of clinical interest, as an increase in oxalate excretion in urine may indicate hyperoxaluria, renal failure, kidney lesions or pancreatic insufficiencies [14]. Calcium oxalate is toxic for human health and, according to the levels of intake, it can cause from throat irritation to coma and death [15]. Oxalate is used in brewing and is usually present in the form of calcium and magnesium oxalate in the bottom of the tanks, which can be dangerous to the health of consumers [16]. With regard to malonate, it is a common compound in living organisms, where it participates in many metabolic routes [17], being an inhibitor of cellular respiration [18]. This dicarboxylate anion is used in the synthesis of other compounds such as barbiturates, artificial flavors, vitamin B1, and vitamin B6. In the case of succinate anion, its intervention in the Krebs cycle, in which it causes the reduction of the FAD coenzyme, representing a key pharmacological drug target is worth highlighting[19]. A mixture of dimethyl succinate, dimethyl glutarate and dimethyl adipate forms Ethasol, which is a biodegradable polar solvent with a low toxicity, making it a good alternative to conventional solvents. Also, adipate derivatives are used as food additives and acidity regulators.

The second aim was to incorporate the new carbazolo[1,2-*a*] carbazole derivative as ionophore into a membrane for an anion-selective electrode and to evaluate its potentiometric response towards oxalate, malonate, succinate, glutarate and adipate anions. To the best of our knowledge, although some ionophore-based ISEs have been published for oxalate [20–23], none has been proposed for malonate, succinate, glutarate and adipate with the exception of an ISE used as chromatographic potentiometric detector for organic acids [24]. However, due to the low working pH of the mobile phase, the potential response of the detector does not correspond to the dianionic forms.

Finally, in order to obtain additional structural information about the complex formed between the ionophore and these anions, ^{1}H NMR and fluorescence studies were carried out. This information includes the nature of the ion–ionophore interactions as well as the calculation of the corresponding binding constants.

2. Experimental

2.1. Apparatus and electrodes

Potentiometric measurements were recorded using a home-made high-impedance data acquisition 16-channel box connected to a personal computer by USB. A Fluka electrode body ISE and an Orion Ag/AgCl double-junction reference electrode (Orion 90-02) containing 10^{-2} M KCl in the outer compartment were used. ^{1}H NMR spectra were recorded on either a Bruker AV200 or a Bruker AV400. Emission spectroscopy experiments were carried out using a Cary Eclipse spectrophotometer.

2.2. Reagents and solutions

All chemicals were of analytical reagent grade and Milli-Q water was used throughout. Organic solvents were dried following the usual protocols.

Polyvinyl chloride (PVC) of high molecular weight, 2-nitrophenyl octyl ether (NPOE), tridodecylmethylammonium chloride (TDMACl) and tetrahydrofuran (THF) were purchased from Fluka.

Potentiometric measurements were carried out using aqueous solutions of the following series of anions used in the form of their sodium salts: perchlorate, salicylate, thiocyanate, iodide, nitrate, bromide, chloride, sulfate, acetate, dihydrogen phosphate, fluoride, hydrogen carbonate, oxalate, malonate, succinate, glutarate and adipate.

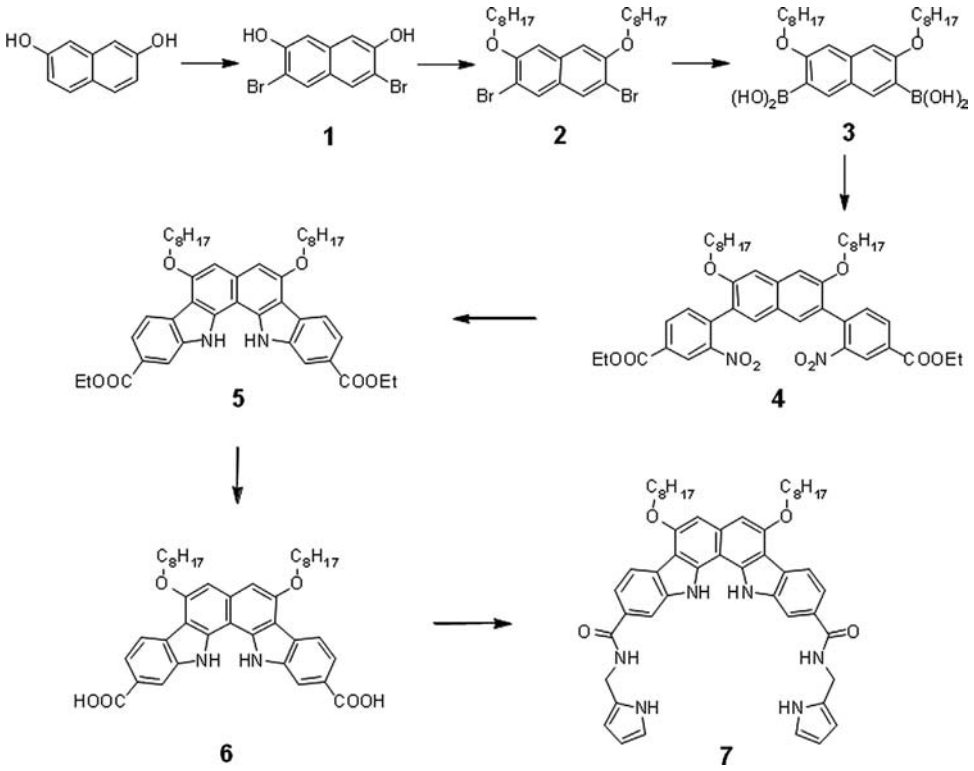

Fig. 1. Synthetic route for 2,11-bis(N-pirrol-2-ylmethylcarbamoyl)-5,8-dioctyloxycarbazolo[1,2-*a*]carbazol.

2.3. Synthesis of the ionophore 2,11-bis(N-pyrrol-2-ylmethylcarbamoyl)-5,8-dioctyloxycarbazolo[1,2–a]carbazole

The synthetic route for 2,11-bis(N-pirrol-2-ylmethylcarbamoyl)-5,8-dioctyloxycarbazolo[1,2-*a*]carbazole is depicted in Fig. 1. The route starts with a regioselective bromination of 2,7-dihydroxynaphthalene in positions 3 and 6. Then, a Williamson etherification with 1-bromooctane was performed to obtain the dibromodioctyloxy derivate, **2**. Next, this compound was transformed into the corresponding diboronic acid, **3**, by treatment with n-butyllithium followed by $B(OEt)_3$ [25]. A subsequent double Suzuki–Miyaura cross-coupling reaction with ethyl 4-iodo-3-nitrobenzoate led to compound **4**, which gave access to the carbazolocarbazole system, **5**, via a Cadogan nitrene insertion. The basic hydrolysis of the ester groups led to the corresponding dicarboxylic acid, **6**. Finally, the reaction of **6** with N, N′-carbonyldiimidazole and 2-aminomethylpyrrol yielded the desired carbazolocarbazole, **7**, as a pale yellow solid.

3,6-Di(4-ethoxycarbonyl-2-nitrophenyl)-2,7-dioctyloxynaphthalene, 4: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.84–0.9 (m, 6H), 1.23–1.25 (m, 20H), 1.45 (t, J=7.2 Hz, 6H), 1.63–1.67 (m, 4H), 3.97 (t, J=6 Hz, 4H), 4.46 (q, J=7.2 Hz, 4H), 7.07 (s, 2H), 7.58 (d, J=8 Hz, 2H), 7.75 (s, 2H), 8.33 (dd, J_1=2 Hz, J_2=8.2 Hz, 2H), 8.66 (d, J=2 Hz, 2H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1 ($2CH_3$), 14.9 ($2CH_3$), 22.6 ($2CH_2$), 25.8 ($2CH_2$), 28.5 ($2CH_2$), 29.1 ($2CH_2$), 29.7 ($2CH_2$), 31.7 ($2CH_2$), 61.8 ($2CH_2$), 68.5 ($2CH_2$), 105.4 (2CH), 123.8 (2C), 125.1 (2CH), 126.7 (C), 129.1 (2CH), 130.6 (2C), 133.0 (2CH), 133.5 (2CH), 136.7 (2C), 137.5 (C), 149.6 (2C), 154.4 (2C), 164.6 (2C=O) ppm; HRMS *m/z* calculated for $C_{44}H_{54}N_2O_{10}$ 771.3851, found 771.3857 $(M+H)^+$; m.p. 89–91 °C.

2,11-Diethoxycarbonyl-5,8-dioctyloxycarbazolo[1,2-*a*]**carbazole, 5:** ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88–0.92 (m, 6H), 1.32–1.41 (m, 22H), 1.66–1.70 (m, 4H), 1.99–2.06 (m, 4H), 4.19–4.40 (m, 8H), 6.84 (s, 2H), 7.97 (d, J=8 Hz, 2H), 8.30–8.34 (m, 4H), 11.15 (s, 2H, NH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1 ($2CH_3$), 14.4 ($2CH_3$), 22.7 ($2CH_2$), 26.3 ($2CH_2$), 29.3 ($4CH_2$), 29.5 ($2CH_2$), 31.9 ($2CH_2$), 60.8 ($2CH_2$), 68.1 ($2CH_2$), 98.7 (2CH), 100.0 (2C), 109.2 (2C), 112.4 (2CH), 120.9 (2CH), 121.5 (2CH), 124.6 (2C), 126.5 (2C), 136.6 (C), 137.0 (C), 138.1 (2C), 154.7 (2C), 168.1 (2C=O) ppm; HRMS *m/z* calculated for $C_{44}H_{54}N_2O_6$ 707.4055, found 707.4035 $(M+H)^+$; m.p. 183–186 °C.

2,11-Dicarboxy-5,8-dioctyloxycarbazolo[1,2–a]**carbazole, 6:** ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.85 (m, 6H), 1.22–1.28 (m, 16H), 1.50–1.58 (m, 4H), 1.96–1.98 (m, 4H), 4.26 (t, J=6 Hz, 4H), 7.16 (s, 2H), 7.87 (d, J=8.4 Hz, 2H), 8.29 (d, J=8.4 Hz, 2H), 8.33 (s, 2H), 12.08 (s, 2H, NH), 12.80 (bs, 2H, COOH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.9 ($2CH_3$), 22.1 ($2CH_2$), 25.7 ($2CH_2$), 28.7 ($4CH_2$), 28.7 ($2CH_2$), 31.2 ($2CH_2$), 67.6 ($2CH_2$), 99.2 (2CH), 99.5 (2C), 108.4 (2C), 112.4 (2CH), 120.7 (2CH), 120.9 (2CH), 125.4 (2C), 125.6 (2C), 136.2 (C), 136.4 (C), 137.8 (2C), 154.2 (2C), 168.1 (2C=O) ppm; HRMS *m/z* calculated for $C_{40}H_{46}N_2O_6$ 651.3429, found 651.3434 $(M+H)^+$; m.p. dec. > 300 °C.

2,11-Di(N-pyrrol-2-yl-methylcarbamoyl)-5,8-dioctyloxycarbazolo[1,2-*a*]**carbazole, 7:** ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83–0.87 (m, 6H), 1.25–1.39 (m, 16H), 1.50–1.60 (m, 4H), 1.90–2.00 (m, 4H), 4.30–4.31 (m, 4H), 4.50–4.58 (m, 4H), 5.95–5.98 (m, 4H), 6.66 (s, 2H), 7.19 (s, 2H), 7.81–7.83 (m, 2H), 8.25–8.30 (m, 4H), 8.91 (s, 2H, NHamide), 10.62 (s, 2H, NHpyrrol), 12.14 (s, 2H, NHcarb) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.0 ($2CH_3$), 22.2 ($2CH_2$), 25.9 ($2CH_2$), 28.8 ($6CH_2$), 31.3 ($2CH_2$), 36.4 ($2CH_2$), 67.6 ($2CH_2$), 99.1 (2CH), 99.8 (2C), 105.8 (2CH), 107.2 (2CH), 108.4 (2C), 110.6 (2CH), 117.1 (2 × CH), 118.8 (2CH), 120.7 (2CH), 124.2 (2C), 129.4 (2C), 129.8 (2C), 135.8 (C), 136.0 (C), 138.0 (2C), 154.0 (2C), 167.0 (2C=O) ppm. HRMS *m/z* calculated for $C_{50}H_{58}N_6O_4$ 807.4592, found 807.4588 $(M+H)^+$; m.p. dec. > 300 °C.

Table 1
Composition of the membranes assayed..

Membrane	Percentage (w/w) of the components in the membrane			
	PVC	NPOE	Ionophore	TDMACl
A	32.9	65.8	1	0.3 (0.5)
B	33.3	66.4		0.3
C	33	66	1	
D	32.4	64.8	2.3	0.5 (0.3)
E	32.8	65.8	0.9	0.5 (0.7)

Molar ratio of TDMACl relative to ionophore is presented in brackets

2.4. Membrane and electrode preparation

The membranes were prepared by dissolving appropriate amounts of the corresponding components in 3 mL of THF. The amounts of the components of the selected membrane were 100 mg PVC (32.9% of the total weight of the membrane), 200 mg NPOE (65.8%), 3.0 mg ionophore (1%) and 1.1 mg TDMACl (0.3%). The compositions of other membranes tested are shown in Table 1. Each cocktail solution was poured into a Fluka glass ring (inner diameter 28 mm, height 30 mm) on a Fluka glass plate, and allowed to settle overnight until total evaporation of THF had occurred, to obtain a thin plastic membrane. A 6-mm-diameter piece was cut out with a punch for ion-selective membranes and incorporated into a Fluka electrode body ISE containing 1×10^{-4} M KCl as internal filling solution. The electrodes were conditioned in water until they reached a constant potential. When not in use, the electrode was kept immersed in water.

2.5. Potentiometric measurements

All potentiometric measurements were performed at room temperature. Dynamic calibrations of the electrodes were made by adding, while stirring, adequate small volumes of the corresponding standard solution of anion sodium salts in 50.0 mL of water. Before each new calibration the electrode was conditioned in water until the original potential was achieved. If the baseline did not fully reach that value, the water used was renewed.

The steady-state potentials were then plotted versus logarithmic values of the corresponding concentrations. When the potentiometric response for the anion was of Nicolski–Eisenman type, data were fitted to the equation:

$$E = E^0 + S \log (C_A + LD) \quad (1)$$

where E^0 is the standard potential of the cell, S is the calibration slope, C_A is the concentration of the corresponding anion and LD is the limit of detection [26]. Due to the basic behavior of dicarboxylate anions, the concentration values of the dianionic forms were calculated from the corresponding analytical concentration and the base dissociation constant.

2.6. Titration experiments

A stock solution of the ionophore was prepared in dimethylsulfoxide ([ionophore]= 4×10^{-3} M for ^{1}H NMR experiments and 2×10^{-5} M for emission spectroscopy experiments). The anions, used as tetrabutyl ammonium salts, were then dissolved with the appropriate volume of the former solution to obtain the correct concentration of the titrant ([anion]=0.16 M for ^{1}H NMR experiments and 0.015 M for emission spectroscopy experiments). Aliquots of the latter solution were added to the solution which contained the receptor, without having to consider any dilution effects on the titrated species.

3. Results and discussion

The carbazolocarbazole system has been demonstrated to be a good anion receptor. Its arch-shape, which defines a binding cavity with two highly preorganized pyrrole rings, facilitates up to four hydrogen bond interactions through the pyrrolic NHs and the CHs at positions 1 and 12 [27]. Accordingly, we have expanded the binding cavity to explore the possibility of hosting larger anions such as dicarboxylates. In this regard, a disubstituted carbazolo [1,2-*a*]carbazole has been synthesized with two appended N-(pyrrole-2-ylmethyl)amide units at the 2 and 11 positions in order to increase the number of interacting sites in the receptor, as described in the Experimental section.

3.1. Potentiometric response towards dicarboxylate anions

The potentiometric responses of the electrodes based on NPOE-plasticized polymeric membranes, containing and not containing ionophore (membranes A and B, Table 1), were obtained towards a series of dicarboxylate anions: oxalate, malonate, succinate, glutarate and adipate (Fig. 2). A plasticizer with a relatively high dielectric constant was used to prevent ion-pair formation in the membrane [28]. As can be seen in Fig. 2, two aspects of the results obtained were of particular note. First, the potentiometric response of the membrane containing ionophore towards all the dicarboxylate anions tested (Fig. 2b) was higher than that obtained with the blank membrane (Fig. 2a). Second, the order of the response obtained for the anions differed between both membranes. The response obtained for the blank membrane displayed the expected trend based on the lipophilicity of the anions since the potentiometric response increased with the number of carbon atoms of the anion (adipate > glutarate > succinate > malonate > oxalate). However, the membrane containing the ionophore showed a different order in the response for the dicarboxylate anions (glutarate > succinate > malonate > adipate > oxalate). As can be seen, the response order was altered in the case of adipate. In short, the incorporation of the carbazolocarbazole derivative into the membrane as ionophore not only produced an alteration in the magnitude of the response of the anions but also in their response order.

The effect of the incorporation of the cationic lipophilic additive to the membrane was evaluated by studying the response of an electrode with a NPOE-plasticized polymeric membrane containing the ionophore but not the cationic additive (membrane C, Table 1). This electrode showed a practically negligible response towards the anions, meaning that the presence of the cationic additive is necessary to display a potentiometric response. It also indicates that the ionophore acts as a neutral carrier as suggested by different authors working with other anion ionophores that act as hydrogen binding compounds [9,20,29].

The influence of the additive/ionophore molar ratio on the membrane composition was studied by recording the potentiometric response of different membranes (A, D and E) towards oxalate (Fig. 3). Changing this ratio from 0.3 to 0.5 had no significant effect on the slope and the limit of detection for oxalate.

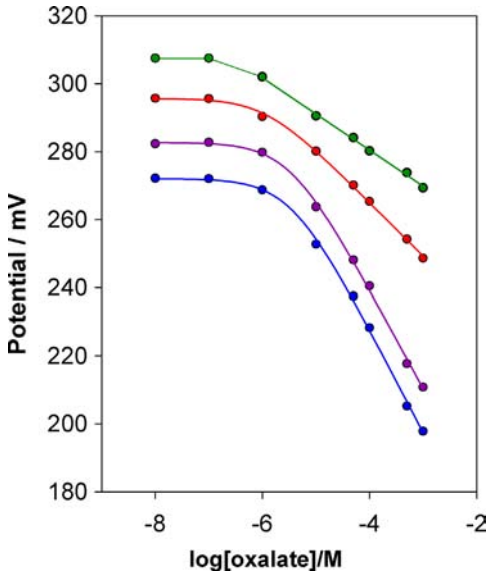

Fig. 3. Calibration graphs obtained using NPOE plasticized polymeric membranes with an additive/ionophore molar ratio of 0.3 (blue), 0.5 (violet), 0.7 (red) and without ionophore (green). (For interpretation of the references to color in this figure , the reader is referred to the web version of this article.)

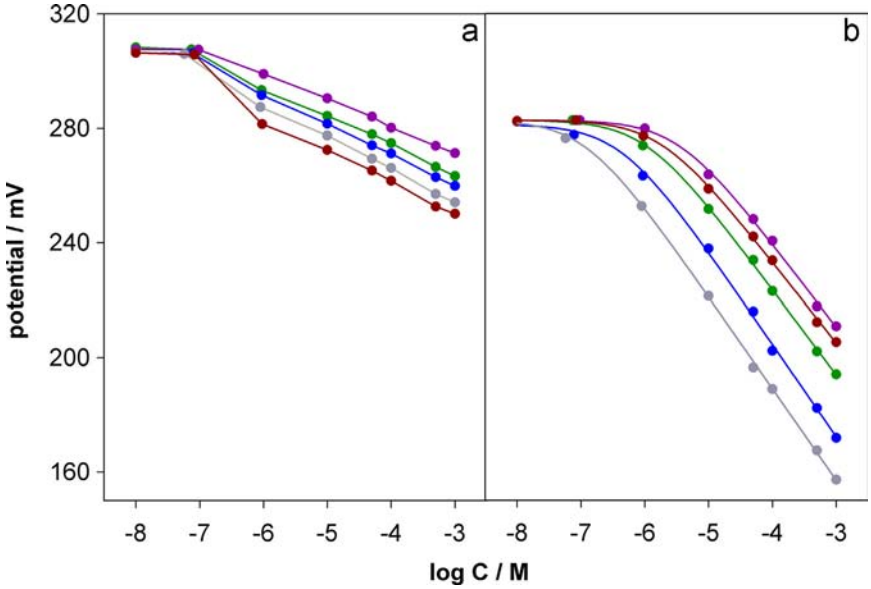

Fig. 2. Calibration graphs obtained using the blank membrane (a) and the membrane containing the ionophore (b) towards oxalate (purple), malonate (green), succinate (blue), glutarate (gray) and adipate (dark red). (For interpretation of the references to color in this figure , the reader is referred to the web version of this article.)

Table 2
Response characteristics of the electrode containing the carbazolocarbazole derivative for oxalate, malonate, succinate, glutarate and adipate.

	Oxalate	Malonate	Succinate	Glutarate	Adipate
S (mV/dec)	−29.1	−29.8	−32.3	−31.7	−28.3
LD (M)	3.3×10^{-6}	1.0×10^{-6}	1.2×10^{-6}	4.4×10^{-7}	1.8×10^{-6}
LLR (M)	5×10^{-6}	2×10^{-6}	3×10^{-6}	5×10^{-7}	3×10^{-6}
Response time (s)[a]	≤15	≤13	≤10	≤9	≤11
Repeatability[b]					
S ± SD (mV/dec)	−29.2 ± 0.3	−29.7 ± 0.2	−32.0 ± 0.4	−31.7 ± 0.3	−28.4 ± 0.4
LD ± SD (M) × 10^6	3.2 ± 0.2	1.3 ± 0.4	0.91 ± 0.04	0.45 ± 0.02	3.2 ± 0.2
Between-day reproducibility[c]					
S ± SD (mV/dec)	−28 ± 1	−28 ± 2	−30 ± 2	−29 ± 2	−27.5 ± 0.8
LD ± SD (M) × 10^6	4.5 ± 1.3	2.2 ± 1.2	3.0 ± 1.8	0.67 ± 0.23	3.7 ± 1.9
Between-membrane reproducibility[d]					
S ± SD (mV/dec)	−29.4 ± 0.2	−30.1 ± 0.4	−29.9 ± 0.5	−30.9 ± 0.8	−29.0 ± 0.7
LD ± SD (M) × 10^6	3.5 ± 0.3	1.1 ± 0.1	1.3 ± 0.1	0.52 ± 0.1	2.8 ± 0.2

SD=standard deviation.
[a] expressed as t_{95} and calculated for the whole concentration range,
[b] n=3,
[c] n=5,
[d] n=2.

The choice of this ratio should take into account the expected lifetime of the membrane. Since the deterioration of the potentiometric response due to the leaching of TDMACl from the membrane to the sample solution occurs earlier with a smaller amount, the 0.5 ratio is more advisable. Increasing the ratio from 0.5 to 0.7 worsened the potentiometric response for oxalate (Fig. 3), which becomes more similar to that obtained with the blank membrane. Taking all this into account, membrane A, with a 0.5 additive/ionophore molar ratio, was selected for further studies.

The response characteristics obtained with the selected membrane are shown in Table 2. A Nernstian response was found for all the dicarboxylate anions and the best response was obtained for glutarate, with a particularly good limit of detection (4.4×10^{-7} M) and a quite good lower limit of the linear range (5×10^{-7} M). The response time was obtained by measuring the time required to reach 95% equilibrium potential after increasing the concentration of the corresponding anion. As can be seen, a fast response time was obtained for all the anions.

Repeatability was studied by making three successive calibrations on the same day for each anion. The between-day reproducibility was evaluated by carrying out five calibrations over a period of one month using the same membrane and with the electrode under continuous work. The between-membrane reproducibility was obtained by making a calibration graph for each anion using two different membranes of the same composition. The mean and standard deviation (s) for the slope (S) and limit of detection (LD) were obtained. The results shown in Table 2 point to a good reproducibility of the calibration parameters, even after one month with the electrode under continuous work. More pronounced changes in slope and limit of detection could be detected after one month.

3.2. Influence of pH

The influence of pH (2.5–12) on the carbazolocarbazole-based electrode was studied by recording the change in the potential response towards oxalate and chloride (a non-basic anion) following the additions of very small aliquots of NaOH and H_3PO_4 solutions of different concentrations (Fig. 4a). As can be seen, the potentiometric response towards chloride is practically independent of the pH, which suggests that the ionophore does not undergo protonation in the membrane within this pH range. As regards the oxalate anion, the potential was practically constant above pH 6, while below this value the potential increased (lower anionic response) as a consequence of the protonation of the oxalate dianion ($pK_{a2}=4.27$). This finding confirms that the ionophore interacts with the dianionic form.

Calibration graphs for oxalate were carried out in different pH buffers and ionic strength adjustors: 0.1 M and 0.01 M Na_2SO_4; 0.05 M and 0.005 M phosphate buffer of pH 8.2; 0.1 M and 0.01 M phosphate buffer of pH 9.1and water (Fig. 4b). No drastic variations in the slope were observed for any of the media used. The LD obtained at the lower concentrations of the different media assayed was slightly higher than in water (2.8×10^{-6}, 9.5×10^{-6}, 4.0×10^{-6} and 3.3×10^{-6} M), while at the higher concentrations an increase of about one decade was obtained (1.5×10^{-5}, 4.7×10^{-5} and 6.2×10^{-5} M).

3.3. Potentiometric response towards other anions

To evaluate the response of the electrode towards other anions, selectivity coefficients were calculated using the separate solution method [30]. Unbuffered solutions were used in these studies to avoid any contribution from the buffer anions [31]. The values obtained for the logarithmic selectivity coefficient were referred to chloride ($\log K^{pot}_{Cl,J}$) and are shown in Fig. 5, together with those obtained for the membrane containing no ionophore for comparative purposes. The higher the $\log K^{pot}_{Cl,J}$, the greater the selectivity towards J over Cl^-.

According to the recommendations of Bakker et al. [32], in order to avoid biased values, the selectivity coefficients should not be calculated for ions which do not show a Nernstian response. Instead of the coefficient, it is sometimes preferable to show the calibration graph only for comparative purposes although apparent selectivity coefficients are also useful for certain comparisons. Calibration graphs for anions that did not display a Nernstian slope, together with that obtained for chloride, are shown in Fig. 6a for the blank membrane and in Fig. 6b for the ionophore-based membrane.

As can be seen in Fig. 5, the incorporation of the carbazolocarbazole derivative as ionophore in the membrane produced a 10^3–10^4 fold increase in the selectivity coefficients for dicarboxylate anions. Moreover, the coefficients for glutarate and succinate overcome the coefficient for chloride.

3.4. ^1H NMR experiments

In order to obtain additional structural information about the type of interaction between the ionophore and the anions under

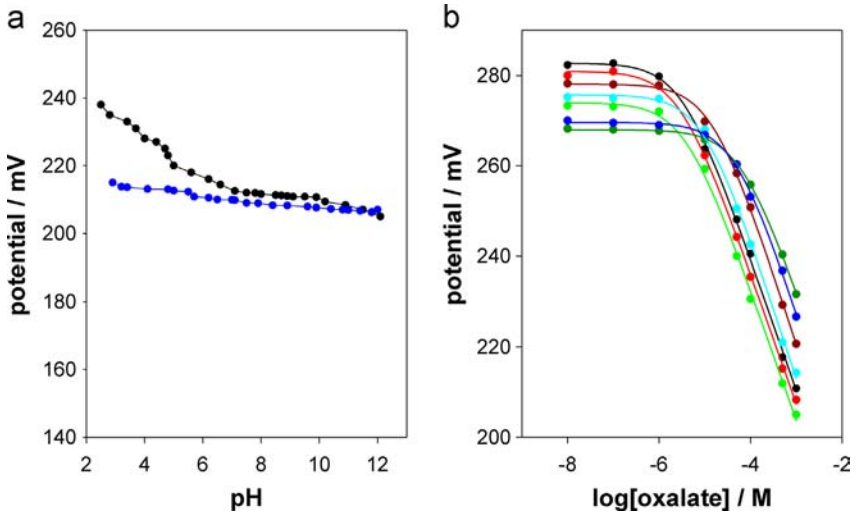

Fig. 4. (a) Effect of pH on the potentiometric responses of ionophore-based membrane in 10^{-3} M oxalate (black) and 10^{-3} M chloride (blue). (b) Calibrations obtained for oxalate in 0.1 M (dark red) and 0.01 M (red) Na_2SO_4; 0.05 M (dark blue) and 0.005 M (blue) phosphate buffer of pH 8.2; 0.1 M (dark breen) and 0.01 M (green) phosphate buffer of pH 9.1 and water (black). (For interpretation of the references to color in this figure , the reader is referred to the web version of this article.)

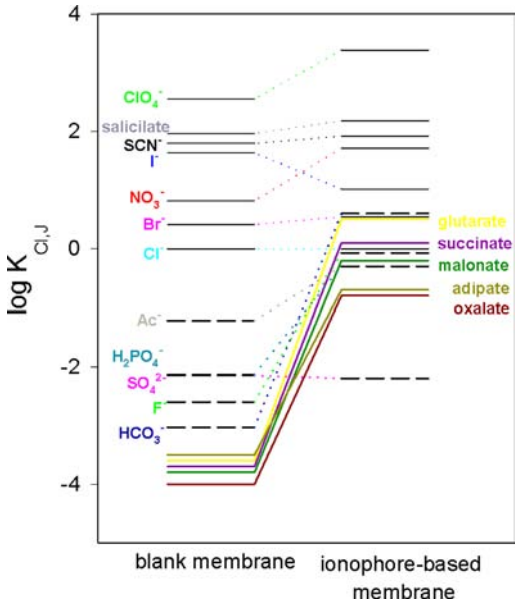

Fig. 5. Logarithm of selectivity coefficients calculated with respect to chloride anion for the blank membrane and for the membrane containing the carbazolocarbazole derivative as ionophore.

evaluation, ^{1}H NMR experiments were performed by titrating the ionophore with the dicarboxylate anions in the form of tetrabutylammonium (TBA) salts in DMSO-d_6. In general all anions showed a clear hydrogen bond interaction with the binding sites in the carbazolocarbazole cavity. In this regard, downfield shifts could be detected for the NH protons as a result of the deshielding effect caused by the polarization of the hydrogen nuclei upon anion binding. This hydrogen bond interaction was observed also for the signals ascribed to the CHs in positions 1 and 12 of the carbazolocarbazole system, the amide NHs and the pendant pyrrole NHs (Fig. S1), supporting the synergistic interaction of the different hydrogen bond donor sites with the dicarboxylate anions.

Nevertheless, the magnitude of the increment in the chemical shift measured for the carbazolocarbazole NHs is larger than that determined for the other hydrogen bond donor sites. This can be related to the strength of the hydrogen bond interaction which is stronger for the carbazolocarbazole NHs, leading to a distinction between a primary binding site represented by the carbazolocarbazole unit and a secondary binding site represented by the pendant substituents. The shape of the binding curves also supports this different binding interaction [33]. Whereas the curves plotted for the carbazolocarbazole NHs show a response immediately after the addition of the first aliquots of the different dicarboxylate anions and reach a plateau, this behavior is not always observed for all the other monitored protons. Conversely two-phase curves with sigmoidal shape or without a saturation plateau were detected for the amide NHs and the pendant pyrrole NHs with certain anions. These dissimilarities between the different interacting sites in the molecule may be due to cooperativity processes or to multiple stoichiometry equilibria which hinder the quantitative analysis of the experimental results. Whatever the case, it should be noted that a qualitative trend in the anion binding affinity could be clearly detected by comparing the titration isotherms of the series of dicarboxylate anions. It is evident that sharper titration profiles were obtained for glutarate and adipate anions (Fig. S2). Moreover, these reached the saturation region at a lower anion concentration than those of shorter dicarboxylates such as succinate, malonate and oxalate. This result would be directly related to the stability of the supramolecular complex, which follows the order glutarate ≥ adipate > succinate > malonate > oxalate.

Although the basicity of the anion [34] may influence the hydrogen bond interaction with the ionophore, the affinity trend clearly indicates that this is not the case for the new carbazolocarbazole, whose binding selectivity seems to be dictated by the appropriate size correspondence between host and guest species. Accordingly, longer dicarboxylate anions such as glutarate and adipate interact better with the different binding sites in the receptor. Conversely, shorter dicarboxylates cannot efficiently bridge the different binding zones in the ionophore structure and show a much weaker interaction.

3.5. Emission spectroscopy experiments

The above described behavior was confirmed by titration experiments followed by emission spectroscopy, in which the fluorescence from the conjugated carbazolocarbazole system allows the complexation equilibrium to be monitored. The emission spectrum of the receptor (Fig. S3a) shows a broad band with a maximum at 431 nm and two shoulders at 411 nm and 524 nm. Fluorescence quenching was detected following dicarboxylate anion complexation, the extent of which was related to the binding affinity. In this regard, the titration

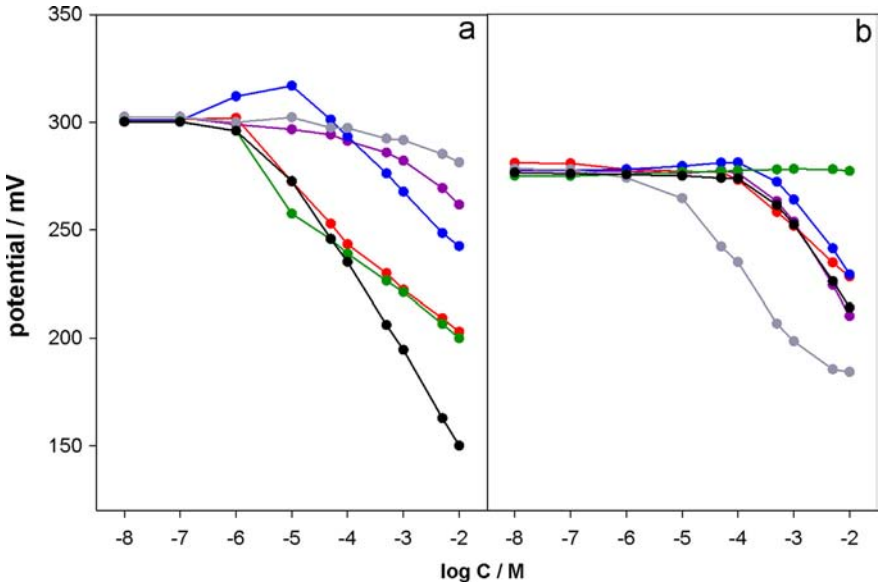

Fig. 6. Calibration graphs obtained for chloride (black), acetate (red), sulfate (green), dihydrogen phosphate (blue), fluoride (purple) and hydrogen carbonate (gray) using the blank membrane (a) and the ionophore-based membrane (b). (For interpretation of the references to color in this figure , the reader is referred to the web version of this article.)

isotherms again pointed to the preferential binding for glutarate anion, followed by adipate, succinate, malonate and oxalate. The binding curves recorded in the more diluted concentration range for the fluorescence spectroscopy fitted well to a 1:1 stoichiometry model (Fig. S3b). Complexation constants, calculated by non-linear regression of the experimental data (data were analyzed with Specfit/32 software) and at λ_{exc}= 370 nm, were 1.10×10^5, 1.62×10^5, 1.86×10^4 and 4.07×10^3 M^{-1} for adipate, glutarate, succinate and malonate, respectively (all the values with a standard deviation lower than 4.0%).

4. Conclusions

The new carbazolocarbazole derivative synthesized in this work behaves as a good ionophore for dicarboxylate anions, especially for glutarate. The observed potentiometric selectivity depends on the good correspondence between the size of the carbazolocarbazole cavity and the length of the dicarboxylate anion, as supported by NMR and fluorescence studies.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.02.022.

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