Published in final edited form as:

Anal Chim Acta. 2007 March 28; 587(2): 247–253.

POTENTIOMETRIC RESPONSE AND MECHANISM OF ANIONIC RECOGNITION OF HETEROCALIXARENE-BASED ION SELECTIVE ELECTRODES

T.V. Shishkanova^{*}, D. Sýkora, J.L. Sessler, and V. Král

Contribution from the Department of Analytical Chemistry, Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic and Department of Chemistry and Biochemistry and Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, Texas 78712-1167

Abstract

The ion selective electrode (ISE)-based potentiometric approach is shown to be an effective means of characterizing the anion recognition sites in the molecular receptor calix[2]pyridino[2]pyrrole (CPP). In particular, potentiometric pH-measurements involving the use of experimental PVC-membranes based on CPP revealed the existence of both mono- and diprotonated forms of the receptor under readily accessible conditions. Based on these analyses, apparent surface protonation constants for this heterocalixarene were found to lie between 8.5–8.9 (pK_{B1}) and 3.3–3.8 (pK_{B2}). CPP was found to interact with targeted anionic analytes based on both coulombic and hydrogen bond interactions, as inferred from varying the kinds of ionic sites present within the membrane phase. Potentiometric selectivity studies revealed that CPP preferred “Y-shaped” anions (*e.g.* acetate, lactate, benzoate) over spherical anions (*e.g.* fluoride and chloride), fluoride over chloride within the set of spherical anions, and the *ortho*- isomer over the corresponding *meta*- and *para*- isomers in the case of hydroxybenzoate (salicylate and congeners). In the context of this study the advantages of potentiometric determinations of acetylsalicylic acid using optimized PVC-membranes based on CPP relative to more conventional PVC-membrane ISEs based on traditional anion exchanger were also demonstrated.

Keywords

Potentiometry; ion selective electrodes; hydroxybenzoate; heterocalixarene; anion recognition

1. Introduction

The challenge of constructing analyte-specific ion selective electrodes (ISEs) is increasingly attracting the attention of supramolecular chemists. This is because such systems could provide a convenient means of characterizing, under interfacial organic-aqueous conditions, the substrate binding characteristics of receptors that might not otherwise be amenable to study in the presence of water due to, *e.g.*, poor aqueous solubility and/or overly weak binding affinities. ISEs are also of obvious interest because they can help translate the chemistry of new substrate binding systems into tools that can be used to recognize selectively various targeted species in the presence of potentially interfering analytes. In the specific case of anion recognition, this

^{*} Corresponding author. Fax: +420-220-444-352; e-mail: tatiana.shishkanova@vscht.cz

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

approach has been explored extensively by Umezawa, Meyerhoff, Simon, Reinhoudt, Schmidtchen and others using a range of receptors including bis-guanidinium [1–2], porphyrins [3], protonated polyamines [4–7], and protonated sapphyrins [8–10], as well as a variety of Lewis acidic systems such as calixarenes [11–15], uranylsalenophenes [16–17], metalloporphyrins [18–22], metallocenes [23], other organometallic derivatives [24–26], and fluorinated compounds [27].

The *meso*-octamethyldichlorocalix[2]pyridino[2]pyrroles (CPPs; Scheme 1) represent a class of macrocycle that should act as anion binding agents. Unlike the better studied calix[4]pyrroles, the CPPs contain both pyrrolic and pyridine sites within their macrocyclic cores. Thus, the putative anion-receptor interactions of CPPs are expected to reflect contributions from both electrostatic and hydrogen bonding interactions in varying degrees depending on the conditions (*e.g.*, degree of protonation, nature of solvent, etc.). In other words, it could be anticipated that under certain conditions the CPPs would act like pyridinium-type receptors and under others, pyrrole-based receptors. Because of this potential duality, it was proposed that studies of appropriately chosen analytes, capable of multiple types of supramolecular interactions, would provide invaluable insights into the fundamental molecular recognition properties of this class of receptor. With such considerations in mind, the salicylate anion and its congeners were selected as test analytes of choice. The carboxylate group and aromatic core present in the salicylate anion makes it potentially capable of entering into ion-pairing, hydrogen bonding, and π -stacking interactions with a putative receptor. Indeed, the salicylate anion has previously been the subject of analysis-based recognition efforts, including ones focused on the selective coordination to tetraphenylporphyrin [20,28–31], phthalocyanine [32–34], and salenophenes [35] metal centers. The supramolecular interaction properties of salicylate have been exploited in the case of anion-exchange- [36–38], calix[4]pyrrol- [39] and guanidinium derivative-based [1] polymeric membranes. In this paper, the interactions of CPP with salicylate anion have been studied using a potentiometric approach. On the basis of these studies the primary recognition sites within the receptor framework, namely the neutral pyrrole NH protons and the protonated pyridinium centers, could be identified and the role of these disparate sites in achieving anion recognition in large measure “deconvoluted” and assessed.

2. Experimental

2.1. Reagents

High molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (NPOE), tridodecylmethylammonium chloride (TDDMACl), sodium tetraphenylborate (NaTPB), tetrahydrofuran (THF) (stored over molecular sieves) and 2[N-morpholino]ethanesulfonic acid (MES) were purchased from Fluka (Switzerland) and Sigma-Aldrich (Germany). Acids, sodium hydroxide, and various inorganic salts (analytical grade) were obtained from Lachema (Czech Republic). The synthesis of CPP has been reported previously [40]. The analgesics used in this study, Aspirin-C (Bayer AG, Leverkusen, Germany), Acylcoffin and Acylpyrin (Slovakofarma, Hlohovec, Slovakia), were purchased in the form of commercial tablets from a local drugstore in Prague, Czech Republic.

2.2. Membranes

Polymeric membranes derived from either TDDMACl or NaTPB and CPP were cast using a conventional method for ISE membrane preparation [41]. In the present study, 0.7 mL of THF was used to dissolve 100 mg of a mixture composed of the active component, CPP, (3 wt.%), of PVC and plasticizer in a 1:2 (w/w) ratio. When indicated, quantities of TDDMACl or NaTPB (specified as mol% relative to CPP) were added (Table 1). The resultant mixtures were poured

into a metallic ring of 16 mm internal diameter resting on a glass plate, and the THF was allowed to evaporate at room temperature.

2.3. Electrodes and EMF measurements

A disk of 10-mm diameter was punched from the ion-selective membrane prepared as described above, glued onto a polymeric ring of 8 mm internal diameter with a PVC/THF paste, and mounted in the electrode body for EMF measurements. An aqueous solution of 10^{-3} M sodium salicylate was used as the inner filling of the electrode. MES buffer was used to obtain the desired experimental pH of 5.5 and 9. Prior to and after the potentiometric experiment, the electrodes were soaked and regenerated in a solution of 10^{-3} M sodium salicylate. All measurements were carried out at 25°C with cells of the following type: Ag; AgCl; 3 M KCl | sample solution | membrane | inner filling solution; AgCl; Ag. The EMF values were measured using a custom made five-channel electrode monitor [42]. The pH response of the experimental electrode was obtained by titrating 0.05 M HCl with NaOH in the absence and presence of sodium salicylate. The pH was monitored by using a glass electrode PHR 01 and a PHI 04 MG ion- and pH-meter (Labio, Czech Republic).

In studies of the potentiometric response and anion selectivity, working solutions of the analytes in question were prepared by diluting concentrated stock solutions. Calibration curves were constructed by plotting the potential vs the logarithm of the concentration of the anion present in the tested solution. Potentiometric selectivity coefficients ($\log K_{I/J}^{Pot}$) were then determined by the separate solution method (SSM) [43], with the primary (I) and interfering (J) ion concentrations being 10^{-2} M. In those membranes where no theoretical potentiometric response was observed, the potentiometric selectivity was estimated using the matched potential method (MPM) [43]. In the MPM, a 10^{-4} M concentration of sodium salicylate was used as the background. The values were calculated from the concentration of the interfering ion, which induced the same amount of potential change as that induced by increasing the concentration of sodium salicylate to 5.0×10^{-4} M.

2.4. Sample analysis

The content of acetylsalicylic acid in commercial analgesic products (Acylpyrin, Acylcoffin, Aspirin-C; please, see 2.1. Reagents) was assayed using both volumetric and potentiometric methods.

The volumetric analysis was based on the following procedure: briefly, five tablets containing acetylsalicylic acid were ground and 0.4 g of the resulting powder was dissolved in 10 ml ethyl alcohol. The solution was then titrated quantitatively with a solution of 0.1 M sodium hydroxide using the color change of phenolphthalein as the end point; the resulting solution was mixed with 30 ml of 0.1 M sodium hydroxide and was heated for 10 min. After cooling at the ambient temperature, the excess sodium hydroxide was neutralized with an appropriate quantity of dilute aqueous sulfuric acid (0.05 M). A blank experiment was carried out in the same manner.

The potentiometric analysis of analgesics was carried out without subjecting acetylsalicylic acid to hydrolysis and using the method of standard addition at pH 5.5 [44].

3. Results and discussion

In the case of CPP, the expected binding sites are the pyrrole and pyridine subunits. Each one is capable of promoting anion recognition, albeit in a somewhat different fashion. For instance, the pyrrole subunits provide NH hydrogen bond donors that are inherently neutral, although nonetheless subject to potential protonation, as has been demonstrated in the case of ISEs based on calix[4]pyrroles [45]. Thus, at least under many conditions, these subunits might be expected

to support anion recognition through rather straightforward $\text{NH}\cdots\text{X}^-$ hydrogen bonding interactions. In contrast, the acid-base properties of the pyridine nitrogen should allow it to bind anionic species through either $\text{N}:\cdots\text{HO}$ hydrogen bonds at higher pH or coulombic $\text{NH}^+\cdots\text{X}^-$ interactions at low pH that could operate independently or in concert with hydrogen bonding interactions. Thus, it was expected that, depending on the specific conditions of use, CPP might act as mixed anion carrier, *i.e.*, either a charged or neutral carrier.

The main goal of the present study was thus to use a potentiometric approach to obtain insights into the nature of the interaction modes potentially operative in CPP anion substrate recognition. Towards this end, salicylic acid was chosen as the test substrate, because 1) it is potentially capable of interacting with the CPP core through a variety of multi-point interactions, 2) earlier studies with other related systems had established that this is a species which gives a good response in receptor-functionalized ISEs [1,28–35,39], and 3) its derivative, acetylsalicylic acid, represents important pharmaceuticals.

3.1. pH effects

Given the potential pH sensitivity of the CPP receptor (as the presumed result of pyridine N: protonation), our initial interest was to define roughly the pH ranges where the mono- and diprotonated forms prevail. The potential of CPP-based membranes were measured in aqueous media as a function of solution pH in the presence and absence of salicylate anion (Fig. 1A). In the absence of salicylate anion, there were pH ranges where a weak (-31 mV/pH unit: pH 2–5) and sub-Nernstian (-47 mV/pH unit: pH 8–9) responses were seen, as well as ones where pH independent behavior was obtained (below pH 2 and at 6–8). The addition of salicylate anion (sodium salt) led to a negative deviation in two pH ranges, namely, 3.5–6.0 (-11 mV/pH unit) and 8–9 (-25 mV/pH unit). This negative deviation is ascribed to the favorable charge-charge interactions between the protonated CPP pyridinium subunits and the salicylate anion at the membrane/sample solution interface. The stepwise decrease in sensitivity observed in passing from the 9–8 to the 6–3 pH regimes is, accordingly, most easily rationalized in terms of the predominant existence of the mono- and diprotonated forms of CPP, respectively.

Recently, Umezawa *et al.* demonstrated that anionic sites (including the sodium tetraphenylborate, NaTPB, employed in the present study) within the membrane phase can play an important role in modulating receptor protonation and thus their interaction with targeted anionic analytes [46]. These effects mostly take place at the membrane/water interface, since anionic sites help attract protons to the interfacial region. As such, they serve not only to enhance receptor protonation, they also help suppress unfavorable charge-charge repulsions between the resulting protonated receptor molecules and protons present at the interface. Since the anionic site used in the present study, NaTPB, is a weak Brönsted base, it itself is not subject to extensive protonation at the interface. Thus, the pH dependences seen in the case of the CPP-containing membranes can be attributed to changes in receptor protonation, presumably at the membrane surface. Consistent with this proposal is the fact that parallel pH dependent profiles were seen for the CPP electrodes in the presence and absence of NaTPB (Fig. 1B).

The above findings lead us to propose that the CPP receptor is protonated within the membrane over the pH range 2–9. Within the context of this global assesment, we propose that specific surface protonation effects might intervene between 8.5–8.9 (pK_{B1}) and 3.3–3.8 (pK_{B2}). Support for this latter hypothesis comes from proton binding studies of a pyridine-strapped 5,12-dioxocyclam-based macrocycle [47]. Meyer *et al.* showed that this elaborated pyridine-containing receptor behaves as a diprotic base with $\text{pK}_{\text{B1}}=8.94(1)$ and $\text{pK}_{\text{B2}}=2.32(9)$. However, in this latter instance the diprotonated species was thought to be stabilized by additional intramolecular hydrogen bonding within the cavity. For the CPP receptor of the present study, such internal hydrogen bonding interactions are not possible in the diprotonated form (all the

core nitrogens are protonated). Accordingly, the relevant pK_{B2} for CPP is expected to fall in the 3.3–3.8 range.

3.2. Effect of ionic additives on the behavior of CPP-based membranes

Taking into account its susceptibility to protonation, we believe the CPP core should be considered as a mixed anionic carrier, namely a positively charged or neutral carrier (Scheme 2) depending on the effective pH. To the extent such a supposition is true, the observed potentiometric selectivity should depend on both the amount and nature of the ionic sites incorporated within the membrane [48–50].

Because of this potential ambiguity, most potentiometric measurements were carried out at pH 5.5, where the receptor and salicylate anion exist predominantly in the form of the respective monocations and monoanions. The potentiometric results obtained under these well-defined conditions are presented in Table 1.

In the PVC membrane, the CPP receptor, neutral in itself, is protonated at the membrane surface and, consequently, recognizes analyte anions in the sample solution (Scheme 2A). The pyridine nitrogen basicity helps establish limits on what are expected to be optimal concentrations of added anionic sites. An excess of added anionic sites (*i.e.*, sodium tetraphenylborate) could compete with the protonated receptor and actually reverse the potentiometric response of a membrane such that it displays a cationic response rather than the expected anionic response. By decreasing the concentration of anionic sites in the membrane phase, the anionic potentiometric response resulting from the receptor protonation is expected to become stronger [50]. Nonetheless, membranes 2C and 2B, made up without and with ~13.8 mol% NaTPB, respectively, both showed sub-Nernstian anionic responses of –35.3 – –34.4 mV/decade from 10^{-5} M.^a However, the addition of ~26.2 mol% NaTPB led to a loss in the anionic response in favor of a cationic response.

The addition of cationic sites into the CPP-containing membrane phase should support salicylate binding *via* a neutral mechanism as outlined in Scheme 2B. In this case, the both pyrrolic and pyridine groups should be involved in the formation of hydrogen bonds. However, the nature of the interactions is different in the case of these two subunits. This is because the anionic (carboxylate) group present on the salicylate anion can interact with the pyrrole NH proton acting as a hydrogen bond donor, whereas it is expected that the pyridine nitrogen atom will act as a hydrogen bond acceptor binding the OH group present in the analyte. In addition to these first order effects, the formation of stacking interactions between the aromatic subunits within the receptor and those present in the analyte might enhance the overall process of receptor-analyte recognition. Consistently with such hypotheses, it was found that the addition of ~17.4 and 49.4 mol% TDDMACl, a known cationic site additive, to CPP-containing membranes at pH 5.5 served to increase the potentiometric sensitivity relative to what was seen in its absence (membranes 2E and 2F, respectively). In fact, at these concentrations of TDDMACl, near-Nernstian response characteristics were seen at analyte concentrations $\geq 10^{-5}$ M.

The potentiometric selectivity, which was seen to be dependent on the kind of lipophilic additive employed, was thought to reflect interference caused by the carboxylate anions. It leads to the presumption that electrode membranes containing CPP would display selectivity for “Y-shaped” anions (*e.g.*, acetate, lactate, benzoate) over spherical ones (*e.g.*, fluoride and chloride). As a test of this hypothesis, a range of species were tested as reflected in Figure 2. As can be inferred from an inspection of this figure, a selectivity sequence was seen that was

^aAnionic impurities are always present in plasticized PVC membranes. Therefore membrane 2C likely contains a small amount of anionic impurities incorporated within it even though it is nominally free of NaTPB.

different from that predicted based on the Hofmeister series (membrane containing only TDDMACl), a finding that is completely consistent with the involvement of specific receptor-analyte interactions in the anion recognition and sensing process. Moreover, the fact that the addition of either anionic or cationic sites had a different effect on the carboxylate/halide discrimination ratio is consistent with the pyrrolic and pyridine subunits providing a different, and hence non-Hofmeister contribution to the recognition process. In particular, the presence of anionic sites was expected to enhance the effect of both coulombic and hydrogen bond interactions and thus lead to the greatest level of generalized anionic interference. In fact, the greatest such interference was observed for membranes containing ~13.8 mol% NaTPB (membrane 2B). In contrast to what is seen with anionic sites, the presence of cationic sites was expected to enhance the discrimination between anionic interferents in accord with the number of receptor-analyte hydrogen bonds that could be stabilized. To a first approximation, this too was seen. However, it should be noted that the interference from fluoride anion was relatively greater than that produced by chloride. Such a difference argues in favor of a cooperative effect, wherein both coulombic and hydrogen bonding interactions contribute to the recognition of such spherical halide anions. Finally, as noted above, once a large excess of cationic sites is added into the membrane phase (membrane 2G), the selectivity pattern begins to resemble that of membranes based only on anion exchangers (cf. membrane containing only TDDMACl; Table 1).

More recently, a new series of calixphyrin oligopyrrolic macrocycles has been proposed for oxoanions recognition [39]. Their structure lies between that of porphyrins and calixpyrroles and, like CPP, consists of two pyrrolic units and two basic nitrogens. As part of our study, we were interested to compare the affinity of these analogues as receptors for oxoanionic species in ISEs. Unfortunately, the potentiometric results reflecting the presumed binding properties of a prototypical monoarylcalix[4]phyrin revealed a pattern of selectivity only at pH 9 (the potentiometric selectivity coefficients were measured by SSM). At this pH, the monoarylcalix[4]phyrin thus appears to behave as a neutral carrier. In contrast, CPP (membrane 2F) displays interference from acetate, lactate, and benzoate, a finding we interpret as meaning that CPP is more sensitive towards carboxy groups and their presence in analytes under these same experimental conditions.

3.3. Effect of positional isomers on potentiometric selectivity

To follow up on the above and to obtain greater insights into the presumed binding modes, isomers of salicylate anion (*ortho*-hydroxybenzoate), namely, *meta*- and *para*-hydroxybenzoate, were studied, at pH 5.5. This series of potentiometric measurements was carried out so as to determine the effect, if any, of the position of the hydroxyl group on the observed potentiometric response. Therefore, for the sake of comparison, selectivity data for a previously reported salicylate electrode based on Aliquat 336S [28] and Sn[TPP]Cl₂ [28] is also included.

It was found that at pH 5.5, ISEs derived from CPP-based membrane displayed a preference for the *ortho*- isomer of hydroxybenzoate (salicylate) relative to the *meta*- and *para*- isomers. For these latter two species, studied in competition with salicylate, the interference gradually decreased with a distance of the hydroxyl group from carboxyl group (Table 2). In other words, the effect of the *para*- isomer was less pronounced than the *meta*- isomer. While this proved specifically true just for the ISEs made up using CPP, there is simply not enough data to tell whether this generalization will hold in the case of ISEs made up from Aliquat 336S and Sn [TPP]Cl₂.

The differences in selectivity seen for the CPP-based electrodes relative to the other ISE receptors included in Table 2 are believed to reflect differences in the intrinsic recognition modes for the receptors in question. In the case of the Aliquat-336S-based membrane system,

recognition is based on nonselective coulombic interactions with the quarternary ammonium salt. Likewise, for the $\text{Sn}[\text{TPP}]\text{Cl}_2$ -based membrane hydroxybenzoate (salicylate) recognition is the result of its selective coordination to the metal center as an axial ligand. In contrast, CPP is capable of recognizing hydroxybenzoates *via* hydrogen bond interactions (*e.g.*, pyrrolic $\text{NH}\dots\text{carboxylic}$, pyrrolic $\text{NH}\dots\text{OH}$, pyridine $\dots\text{HO}$), as well as coulombic effects, especially pyridinium $\text{NH}^+ \dots ^-\text{O}_2\text{C}$ salt bridges. When these effects can be made to be synergetic, as in the case of salicylate binding, the selectivity for one particular regioisomer would be expected to be particularly high, a finding that is very much in accord with the experimental findings.

3.4. Analytical application

Acetylsalicylic acid is one of the best known and most widely used salicylate derivatives. Based on its structure, and its ability to interact with CPP through more than one recognition mode, it was anticipated that ISEs containing this latter receptor would prove particularly sensitive. As a corollary of this hypothesis, it was anticipated that the ISEs of this study would allow for the detection of acetylsalicylic acid present in various commercial analgesic products. Towards this end, three commercially available analgesic products containing acetylsalicylic acid were tested. The results obtained by potentiometric analysis using the CPP-based electrodes of this study proved to be in good agreement with those recorded using standard volumetric procedures (Table 3). In accord with the mechanistic rationale given above, the CPP-based PVC-membranes proved more accurate than those derived from just TDDMACl (*cf.* last entry in Table 1).

4. Conclusions

The potentiometric measurements provided insights into the fundamental recognition processes that underlie the observed potentiometric response and the basic chemical properties of CPP. For instance, pH-dependence experiments served to support the notion that the apparent protonation constants of this particular heterocalixarene might lie between 8.5–8.9 (pK_{B1}) and 3.3–3.8 (pK_{B2}). Parallel studies, that involved varying the kinds of ionic sites present within the membrane, served to demonstrate that both the charged and neutral forms of this particular heterocalixarene display an inherent affinity for “Y-shaped” anions (*e.g.* acetate, lactate, benzoate) over spherical anions (*e.g.* fluoride and chloride). The fact that the level of this discrimination could be made to vary depending on whether anionic or cationic sites were added to the membrane, lends credence to the conclusion that both hydrogen bonding and coulombic interactions (involving the pyrrolic and pyridine units in CPP) contribute to the recognition process and that the relative importance of these disparate effects varies with the experimental conditions. In contrast to several other ISEs based on other kinds of synthetic receptors, the membranes containing CPP proved capable of discriminating between the three positional isomers of salicylate (*i.e.*, *ortho*-, *meta*-, and *para*-hydroxybenzoate). Given this selectivity and the fact that acetylsalicylic acid could be detected in commercial pharmaceutical products, leads us to suggest that CPP and ISEs derived from it could have a role to play in pharmaceutical analysis, including in high-throughput or sensor array systems, such as those embodied in so-called “electronic tongues”.

Acknowledgements

This research was supported by the Ministry of Education, Youth, and Sports of the Czech Republic (MSM 6046137307) and by the National Institutes of Health (GM 58907 to J.L.S.). Authors thank M.Sedláček for technical assistance.

References

1. Hutchins RS, Bansal P, Alajarin M, Molina P, Vidal A, Bachas LG. *Anal Chem* 1997;69:1273.
2. Fibbioli M, Berger M, Schmidtchen FP, Pretsch E. *Anal Chem* 2000;72:156. [PubMed: 10655648]

3. Amemiya S, Bühlmann P, Umezawa Y, Jagessar RC, Burns DH. *Anal Chem* 1999;71:1049.
4. Umezawa Y, Kataoka M, Takami W, Kimura T, Koike T, Nada H. *Anal Chem* 1988;60:2392. [PubMed: 3239805]
5. Kataoka M, Naganawa R, Odashima K, Umezawa Y, Kimura E, Koike T. *Anal Lett* 1989;22:1089.
6. Tohda K, Tange M, Odashima K, Umezawa Y, Furuta H, Sessler JL. *Anal Chem* 1992;64:960. [PubMed: 1320353]
7. Naganawa R, Radecka H, Kataoka M, Tohda K, Odashima K, Umezawa Y, Kimura E, Koike T. *Electroanal* 1993;5:731.
8. Tohda K, Naganawa R, Xiao ML, Tange M, Umezawa K, Odashima K, Umezawa Y, Furuta H, Sessler JL. *Sensor Actuat B-Chem* 1993;14:669.
9. Odashima K, Naganawa R, Radecka H, Kataoka M, Kimura E, Koike T, Tohda K, Tange M, Furuta H, Sessler JL, Yagi K, Umezawa Y. *Supramol Chem* 1994;4:101.
10. Lin XM, Umezawa K, Tohda K, Furuta H, Sessler JL, Umezawa Y. *Anal Sci* 1998;14:99.
11. Visser HC, Rudkevich DM, Verboom W, de Jong F, Reinhoudt DN. *J Am Chem Soc* 1994;116:11554.
12. Wroblewski W, Brzozka Z. *Anal Chim Acta* 1996;326:163.
13. Wroblewski W, Malinowska E, Brzozka Z. *Electroanal* 1996;8:75.
14. Gupta VK, Ludwig R, Agarwal S. *Anal Chim Acta* 2005;538:213.
15. Lee HK, Oh H, Nam KC, Jeon S. *Sensor Actuat B-Chem* 2005;106:207.
16. Wroblewski W, Brzozka Z, Rudkevich DM, Reinhoudt DN. *Sensor Actuat B-Chem* 1996;37:151.
17. Antonisse MMG, Snellink-Ruel BHM, Ion AC, Engbersen JFJ, Reinhoudt DN. *J Chem Soc Perkin Trans* 1999;2:1211.
18. Bühlmann P, Pretsch E, Bakker E. *Chem Rev* 1998;98:1593. [PubMed: 11848943]
19. Steinle ED, Schaller U, Meyerhoff ME. *Anal Sci* 1998;14:79.
20. Malinowska E, Niedziółka J, Roniecka E, Meyerhoff ME. *J Electroanal Chem* 2001;514:109.
21. Gorski L, Malinowska E, Parzuchowski P, Zhang W, Meyerhoff ME. *Electroanal* 2003;15:1229.
22. Mitchell-Koch JT, Pietrzak M, Malinowska E, Meyerhoff ME. *Electroanal* 2006;18:551.
23. Hisamoto H, Siswanta D, Nishihara H, Suzuki K. *Anal Chim Acta* 1995;304:171.
24. Midgley D. *Anal Chim Acta* 1986;182:91.
25. Rothmaier M, Schaller U, Morf WE, Pretsch E. *Anal Chim Acta* 1996;327:17.
26. Shahrokhian S, Amini M, Kia R, Tangestaninejad S. *Anal Chem* 2000;72:956. [PubMed: 10739198]
27. Siswanta D, Takenaka J, Suzuki T, Sasakura H, Hisamoto H, Suzuki K. *Chem Lett* 1997;26:195.
28. Chaniotakis NA, Chasser AM, Park SB, Meyerhoff ME. *Anal Chem* 1989;61:566. [PubMed: 2729591]
29. Kibbery CE, Park SB, DeAdwyer G, Meyerhoff ME. *J Electroanal Chem* 1992;335:135.
30. Badr IH, Meyerhoff ME, Hassan SSM. *Anal Chim Acta* 1996;321:11.
31. Shahrokhian S, Hamzehloei A, Bagherzadeh M. *Anal Chem* 2002;74:3312. [PubMed: 12139034]
32. Li JZ, Pang XY, Gao D, Yu RQ. *Talanta* 1995;42:1775.
33. Leyzerovich NN, Shvedene NV, Blikova YN, Tomilova LG, Pletnev IV. *Electroanal* 2001;13:246.
34. Shahrokhian S, Jannatrezvani MJ, Khajehsharifi H. *Anal Lett* 2005;38:1221.
35. Shahrokhian S, Amini MK, Kia R, Tangestaninejad S. *Anal Chem* 2000;72:956. [PubMed: 10739198]
36. James HJ, Carmack G, Freiser H. *Anal Chem* 1972;44:856.
37. Choi KK, Fung KW. *Anal Chim Acta* 1982;138:385.
38. Rover JL, Garcia CAB, de G, Neto O, Kubota LT, Galembeck F. *Anal Chim Acta* 1998;366:103.
39. Bernátková M, Andrioletti B, Král V, Rose E, Vaissermann J. *J Org Chem* 2004;69:8140. [PubMed: 15527308]
40. Král V, Gale PA, Anzenbacher PJ, Jursikova K, Lynch V, Sessler JL. *Chem Commun* 1998;9.
41. Král V, Sessler JL, Shishkanova TV, Gale PA, Volf R. *J Am Chem Soc* 1999;121:8771.
42. Beznová H, Volf R, Král V, Sessler JL, Try AC, Shishkanova TV. *Anal Bioanal Chem* 2003;375:1193. [PubMed: 12733038]
43. Umezawa Y, Umezawa K, Sato H. *Pure Appl Chem* 1995;67:508.

44. Koryta, J.; Štulík, K. Ion-selective electrodes. Academia; Prague: 1984.
45. Piotrowski T, Radecka H, Radecki J, Depaetere S, Dehaen W. *Electroanal* 2001;13:342.
46. Umezawa K, Tohda K, Lin XM, Sessler JL, Umezawa Y. *Anal Chim Acta* 2001;426:19.
47. Meyer M, Fremont LT, Tabard A, Espinosa E, Vollmer GY, Guillard R, Dory Y. *New J Chem* 2005;29:99.
48. Schaller U, Bakker E, Pretsch E. *Anal Chem* 1995;67:3123.
49. Amemiya S, Bühlmann P, Pretsch E, Rusterholz B, Umezawa Y. *Anal Chem* 2000;72:1618. [PubMed: 10763261]
50. Radecka H, Tohda K, Umezawa Y. *Anal Sci* 2001;17:1221. [PubMed: 11990601]

Figure 1.
pH dependence of the potentiometric response for
A) CPP-based membrane in the absence (●) and presence (○) of 10^{-3} M sodium salicylate.
B) CPP-based membrane in the absence (●) and presence (△) of anionic sites (13.8 mol% NaTPB relative to receptor).

Figure 2.

Plot showing the potentiometric anion selectivity, $\log K^{\text{Pot}}_{\text{Salicylate, J}}$,^{a)} of the CPP-based membranes containing various concentrations of anionic and cationic sites at pH 5.5. (F^- (●), Cl^- (○), benzoate (■), lactate (□), acetate (◆))

^{a)} All values of the potentiometric selectivity coefficients were obtained using MPM, as detailed in *Experimental*.

Scheme 1.
meso-octamethyldichlorocalix[2]pyridino[2]pyrrole.

(A) Charged carrier mechanism **(B) Neutral carrier mechanism****Scheme 2.**

Possible mechanism of the potentiometric response seen for CPP-based membranes, showing how this receptor system can act as either a singly charged (A) or neutral (B) carrier at pH 5.5 (X^- , analyte anion; R^- and R^+ are added lipophilic anionic and cationic sites, respectively).

Table 1

Composition of membranes based on CPP in *o*-NPOE/PVC (2:1) and their potentiometric properties towards salicylate anions.

Membrane	Composition, wt % (mol % relative to receptor)			Sensitivity, mV/ decade	Linear range, M
	CPP	NaTPB	TDDMACl		
2A	3.0	0.52 (26.4)		+53.0 ± 2	10 ⁻² – 10 ⁻¹
2B	3.0	0.26 (13.8)		-34.6 ± 2	10 ⁻⁵ – 10 ⁻¹
2C	3.0			-35.3 ± 2	10 ⁻³ – 10 ⁻¹
2D	3.0		0.29 (8.8)	-53.2 ± 0.6	10 ⁻³ – 10 ⁻¹
2E	3.0		0.57 (17.4)	-54.2 ± 0.5	10 ⁻⁵ – 10 ⁻¹
2F	3.0		1.63 (49.4)	-58.3 ± 0.5	10 ⁻⁵ – 10 ⁻¹
2G	3.0		4.94 (150)	-53.3 ± 0.5	10 ⁻⁵ – 10 ⁻¹
TDDMACl			3.0	-53.3 ± 0.7	10 ⁻⁵ – 10 ⁻¹

Table 2

The potentiometric selectivity coefficients, $\log K_{O\text{-Hydroxybenzoate}, J}^{Pot(a)}$, of salicylate electrodes prepared with different membrane active components.

Interfering anion	Aliquat 336S ^{b)}	Sn[TPP]Cl ₂ ^{c)}	CPP ^{d)}
Acetate	<-2.70	-3.9	-2.10
Lactate	-2.2	-4.0	-2.2
Benzoate	-1.15	-1.4	-1.3
<i>meta</i> -hydroxybenzoate	-1.34	-1.10	-2.51
<i>para</i> -hydroxybenzoate	-1.30	-1.00	-3.32

^{a)} All values of the potentiometric selectivity coefficients were obtained using the SSM, with the primary (I) and interfering (J) ion concentrations being 10^{-2} M in 0.05 M MES, pH 5.5.

^{b)} Aliquat 336S membranes were prepared with 63 wt.% n-dibutyl phthalate, 30 wt.% PVC and 7 wt.% Aliquat, as detailed in ref [28].

^{c)} Sn[TPP]Cl₂-based membranes were prepared with 66 wt.% DBS, 33 wt.% PVC, and 1 wt.% carrier, as detailed in ref [28].

^{d)} Membrane composition corresponded to that of membrane 2F (Table 1).

Table 3

Results of the determination of acetylsalicylic acid in commercial analgesic products.

Tablet sample (label value, mg/ tablet)	Acetylsalicylic acid (% of the nominal value)		
	Potentiometric analysis		Volumetric analysis
	CPP-membrane	TDDMACI-membrane	
Acylpyrin (500)	99.6 ± 4.4	105.4 ± 4.4	95 ± 4.4
Acylcoffin (450)	102 ± 4.2	106 ± 6.2	102 ± 5.8
Aspirin-C (400)	99.5 ± 4.8	107 ± 5.5	–