# Potentiometric Evaluation of Solvent Polymeric Carbonate-Selective Membranes Based on Molecular Tweezer-Type Neutral Carriers

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**Potentiometric properties of the ion-selective electrodes) based on highly plasticized PVC membranes doped with the carbonate-selective cholic acid (CA) derivatives have been measured. The carbonate-selective neutral carriers have been prepared by coupling one to three trifluoroacetobenzoyl (TFAB) groups to a cholic acid derivative which has three hydroxyl linkers lining on the C3, C7, and C12 positions of its rigid steroidal ring structure. The membranes based on cholic acid derivatives with two TFABs [3,7-bis(TFAB)CA, 3,12-bis(TFAB)CA, and 7,12-bis(TFAB)CA] exhibited remarkably improved carbonate selectivity, indicating that the bis(TFAB)CAs behave like molecular tweezers for the carbonate ion. For example, 3,12-bis(TFAB)CA resulted in 10−300-fold-enhanced carbonate selectivity over other anions (e.g., salicylate, $ClO_4^-$, $SCN^-$, $HPO_4^{2-}$, $NO_3^-$, $NO_2^-$, $Br^-$, and $Cl^-$) compared to that of the neutral carriers with a single TFAB group. The distances between the carbonate binding centers of bis(TFAB)CAs, i.e., the carbonyl carbons of the two TFAB groups, are in the 7.3−7.9-Å range at the AM1 level semiempirical calculation, which is too far for the carbonate ion to form direct covalent bonding. The fast atom bombardment mass spectra of bis(TFAB)CAs show that significant fractions of the compounds are either mono- or dihydrated before complexing the carbonate ion. These findings seem to suggest that bis(TFAB)CAs recognize the incoming carbonate ion by forming both covalent and hydrogen bonding between the hydrated and unhydrated TFAB groups. The analytical utility of the carbonate-selective electrode based on 3,12-bis(TFAB)-CA has been demonstrated by measuring the total carbon dioxide in human serum in the presence of lipophilic anion interferents, e.g., salicylate.**

Accurate and rapid determination of total carbon dioxide ($TCO_2$) in physiological fluids, seawater, and various industrial samples is one of the very challenging analytical problems.[1−4] It is commonly measured with a Severinghaus-type potentiometric gas sensor after acidifying the sample solution below pH 5.5.[5] Alternatively, the $TCO_2$ in an aqueous sample could be determined with a polymer membrane-based carbonate-selective electrode in alkaline pH conditions.[6] Of the two devices, the latter attracts considerable attention because it is not only easier to fabricate and miniaturize but also provides much faster response and recovery times than the former.

The key element of a carbonate-selective electrode is the highly plasticized PVC membrane containing a trifluoroacetophenone (TFAP) derivative (e.g., trifluoroacetyl-*p*-butylbenzene or -*p*-decylbenzene; TFABB or TFADB) as a neutral carrier and a lipophilic quaternary ammonium salt (e.g., tridodecylmethylammonium chloride; TDMACl) as a mobile cationic site.[6−11] Its potentiometric properties are governed by the carbonate-binding ability of the TFAP derivative, the relative composition between the neutral carrier and cationic sites, the type of polymer matrix employed, and the type of dilution buffer. Simon and co-workers and Sokalski et al. attempted to improve the carbonate selectivity of the membranes by employing various TFAP derivatives that have different substituents in the para position.[11,12] However, the use of TFAP derivative-based PVC membranes has been limited in practice because they exhibit a large interfering response to lipophilic anions (e.g., salicylate), especially in clinical analysis.[6,9,11] Optimizing the ratio between the TFAP derivative and TDMACl doped in a PVC membrane, the carbonate selectivity of the resulting electrode could be increased substantially but not high enough to use for serum or seawater analysis.[13] We recently showed that the combined use of carbonate-selective membranes formulated with an alternative polymer matrix (e.g., silicone rubber) and a high-pH dilution buffer system could effectively

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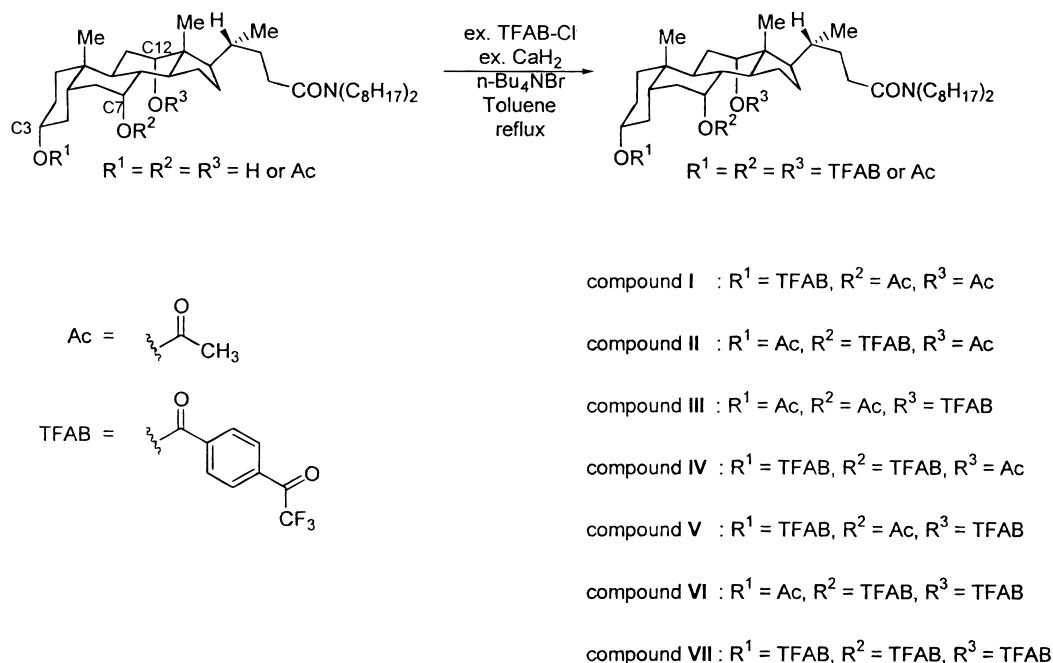
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**Figure 1.** Schemes for the synthesis of cholic acid-based trifluoroacetophenone derivatives and their structures.

suppress the salicylate interference in the measurement of $TCO_2$ in human serum.[14,15] However, it is not easy to come by such a subtle combination.

To improve the practical utility of carbonate-selective electrodes in the analysis of clinical samples and seawater, we need new neutral carriers that can provide enhanced carbonate selectivity, especially over a high concentration (100–600 mM) of chloride, several lipophilic anions (e.g., salicylate, thiocyanate, and iodide), and other hydrophilic anions. Receptors with such characteristics may be designed based on the known carbonate recognition mechanism of TFAP derivatives. Meyerhoff et al. showed that the TFABB normally forms a stable 2:1 adduct with the carbonate ion in the presence of countercations (e.g., tridodecylmethylammonium; $TDMA^+$).[10,13] Their observation may lead to an assumption that the carbonate-selective ionophores with two adjacent TFAP groups would provide enhanced carbonate selectivity, behaving like a molecular tweezers for the carbonate ion. Our recent study, however, indicated that the binding energy between the carbonate ion and the two TFAP groups, if they are floppily linked to an alkyl chain or phenyl, is not enough to overcome the steric repulsion between them.[13] It prompted us to consider molecules that have a rigid carbonate-binding pocket formed by two or more TFAP groups. One plausible strategy was to attach TFAP groups to a rigid platform molecule, e.g., cholic acid.[16,17]

Cholic acid has three hydroxyl linkers at the C3, C7, and C12 carbons, which are lined in the same direction, approximately parallel and about 4.6–6.1 Å apart from each other on the conformationally rigid steroidal ring structure. Substituting molecule-recognizing groups for those hydroxyl groups, it is possible to synthesize various kinds of molecular tweezers based on rigid cholic acid platform.[16] In particular, considering that the distance between the two methyl carbons of dimethyl carbonate is ~4.8 Å, it is expected that the cholic acid derivatives substituted with two or three TFAP units (e.g., trifluoroacetylbenzoyl group; TFAB) may serve as a highly selective molecular tweezers for the carbonate ion. Thus, a series of cholic acid-based TFAP derivatives shown in Figure 1 has been synthesized and their carbonate binding ability has been examined.[17] The UV spectral changes observed for the compounds with two TFAB groups (**IV–VI**) dissolved in dichloromethane, the drastic decrease of the 260-nm peak with increase of the 230-nm peak upon contacting carbonate-containing aqueous solution, indicated that the derivatives effectively bind the carbonate ion.[17] However, no experimental evidence that the cholic acid derivatives with two or three TFAP groups indeed result in the enhanced carbonate selectivity has been provided yet.

In this contribution, we demonstrate that the new compounds synthesized on the basis of the known molecular recognition mechanism of the TFAP group do result in neutral carriers with greatly enhanced carbonate selectivity. To this end, we constructed the PVC membrane-based ion-selective electrodes (ISEs) with the seven cholic acid-based TFAP derivatives shown in Figure 1 and examined their potentiometric properties, i.e., carbonate selectivity over various anions and the response slopes. It is also shown that the ISEs based on cholic acid-based TFAP derivatives could be used to determine the $TCO_2$ in serum even in the presence of a high level of salicylate interference.

## EXPERIMENTAL SECTION

**Reagents.** Poly(vinyl chloride) (PVC), TDMACl, and bis(2-ethylhexyl) adipate (DOA) were purchased from Fluka Chemie AG (Buch, Switzerland). TFADB was a product from Trans World Chemicals (Rocksville, MD). Compounds **I–VII**, *N,N*-dioctyl-7α,-12α-diacetoxy-3α-(4-trifluoroacetylbenzoxy)-5β-cholan-24-amide (**I**

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or 3-(TFAB)CA), $N,N$-dioctyl-3$\alpha$,12$\alpha$-diacetoxy-7-(4-trifluoroacetyl-benzoxy)-5$\beta$-cholan-24-amide (**II** or 7-(TFAB)CA), $N,N$-dioctyl-3$\alpha$,-7$\alpha$-diacetoxy-12$\alpha$-(4-trifluoroacetylbenzoxy)-5$\beta$-cholan-24-amide (**III** or 12-(TFAB)CA), $N,N$-dioctyl-12$\alpha$-acetoxy-3$\alpha$,7$\alpha$-bis(4-trifluoro-acetylbenzoxy)-5$\beta$-cholan-24-amide (**IV** or 3,7-bis(TFAB)CA), $N,N$-dioctyl-7$\alpha$-acetoxy-3$\alpha$,12$\alpha$-bis(4-trifluoroacetylbenzoxy)-5$\beta$-cholan-24-amide (**V** or 3,12-bis(TFAB)CA), $N,N$-dioctyl-3$\alpha$-acetoxy-7$\alpha$,12$\alpha$-bis(4-trifluoroacetylbenzoxy)-5$\beta$-cholan-24-amide (**VI** or 7,12-bis-(TFAB)CA), and $N,N$-dioctyl-3$\alpha$,7$\alpha$,12$\alpha$-tris(4-trifluoroacetylbenz-oxy)-5$\beta$-cholan-24-amide (**VII** or 3,7,12-tris(TFAB)CA), shown in Figure 1 were synthesized and purified as described in ref 17. Tris(hydroxymethyl)aminomethane was obtained from Sigma Chemical Co. (St. Louis, MO). Lyophilized human serum were obtained from Nissui Pharmaceutical Co. (Tokyo, Japan) and used after dissolving them with 5 mL of water. All other chemicals used were analytical reagent grade. Standard solutions and buffers were prepared with freshly deionized water (resistivity, 18 MΩ·cm) and stored under nitrogen atmosphere before use.

**Preparation of Electrodes and Their Potentiometric Evaluation.** The carbonate-selective membranes were prepared from the mixture composed of 8.3 mg of a TFAP derivative (TFADB and **I**−**VII**), 60 mg of PVC, 2 mg of TDMACl, 100 $\mu$L of DOA, and 300 $\mu$L of ethyl acetate. The cocktail solutions dissolved in 0.5 mL of THF were then poured into a glass ring (i.d. 22 mm) placed on a slide glass and dried over a day at room temperature. Small disks were punched from the cast films and mounted in Philips electrode bodies (IS-561; Glasblaserei Möller, Zürich, Switzerland). For all electrodes, a mixture containing NaH$_2$PO$_4$ (0.1 M), Na$_2$HPO$_4$ (0.1 M), and NaCl (0.01 M) was used as the internal reference electrolyte. Potentiometric evaluation of the electrodes was carried out as described previously using a static arrangement: potential differences between the ISEs and the Orion sleeve-type double-junction Ag/AgCl reference electrode (model 90-02) were measured using an IBM AT-type computer equipped with a homemade high-impedance input 16-channel analog-to-digital converter.

The dynamic response curves were obtained by adding standard solutions to 200 mL of magnetically stirred background electrolyte (0.1 M Tris-H$_2$SO$_4$, pH 8.6) every 100 s to vary the concentrations of each ionic species stepwise from $10^{-6}$ to $10^{-1}$ M, and the potential measurements were taken every second at room temperature. The beaker containing electrodes and sample solution was covered with a specially designed top to minimize the atmospheric exposure. Taking equilibrium emf values at the plateau of dynamic curves made calibration plots. Activities of carbonate and other anions (e.g., Cl$^-$, Br$^-$, NO$_3^-$, NO$_2^-$, SCN$^-$, salicylate, and ClO$_4^-$) in 0.1 M Tris-H$_2$SO$_4$ were calculated according to the method given in the refs 18 and 19.

To examine the analytical utility of the ISEs developed in this work, the potentiometric step responses of the carbonate-selective electrode to 10 times-diluted serum samples containing 25 mM carbon dioxide species and 0−3 mM salicylate were measured.

Semiempirical calculations were made using Chem3D program (CambridgeSoft, MA): initial geometries of the **I**−**VII** without a dioctyl group were optimized first with the MM2 calculation and further to the final structure with the AM1 method.

## RESULTS AND DISCUSSION

The seven cholic acid derivatives, **I**−**VII**, shown in Figure 1 were synthesized by selective mono- or diacetylation of the three hydroxyl groups of the dioctyl amide cholic acid and trifluoro-acetylbenzoylation of the remaining hydroxyl groups as described in Figure 1. The dioctyl amide group was introduced to the original cholic acid to increase the lipophilicity of the resulting compounds. The details of the synthesis and spectroscopic identification of the products are given in ref 17. **I**−**VII** may be divided into two groups: **IV**−**VII**, which have two and three TFAB groups, were designed as molecular tweezers for the carbonate ion, while **I**−**III** were prepared to examine the equivalence of each TFAB group substituted to the C3, C7, and C12 positions of the dioctyl amide cholic acid.

The highly plasticized PVC membranes containing **I**−**VII** and TFADB were prepared to compare their potentiometric behavior: the choice of plasticizer, DOA, and the amount of the neutral carriers, 8.3 mg, were based on our previous studies.[13,20] The amount of TDMACl (2 mg) used is about 40−50 mol % with respect to that of **I**−**VII** doped in the membrane, which is known to result in the maximum carbonate selectivity for most TFAP derivative-based membranes.[13] We did not carry out extensive potentiometric performance optimization study, as the primary aim of this work was to evaluate the newly prepared carbonate-selective neutral carriers.

Figure 2 shows the carbonate selectivity of the electrodes based on TFADB and **I**−**VII** over other anions. The selectivity coefficients were evaluated according to the matched potential method suggested by Gadzekpo and Christian; the $K_{CO_3^{2-},j}^{pot}$ values were the activity ratio of the carbonate ($a_{CO_3^{2-}}$) and the interfering anion $j$ ($a_j$), which gives the same potential change from the background potential in a reference solution:[21] In case the electrode exhibited larger potentiometric responses to an interfering ion than to carbonate, the $a_j$ value was determined from the matching potential resulting from the carbonate activity $a_{CO_3^{2-}} = 4.32 \times 10^{-4}$ M [corresponding to $3 \times 10^{-2}$ M NaHCO$_3$ (concentration unit) in 0.1 M Tris-H$_2$SO$_4$, pH 8.6]. In opposite cases, the $a_{CO_3^{2-}}$ value was estimated from the matching potential induced by a 0.1 M interfering ion. Although there are some controversy about the theoretical validity of the matched potential method (MPM), the MPM is known to provide practical guidance for the selectivity of unequally charged ions.[22,23]

It is seen from Figure 2 that the electrodes based on **I**−**III** provide similar potentiometric performance (in terms of their carbonate selectivity, response slopes, and detection limits), but slightly improved over that of the TFADB-based electrode. This result suggests that the carbonate recognition ability of a single TFAB group does not depend on its substituted site, confirming

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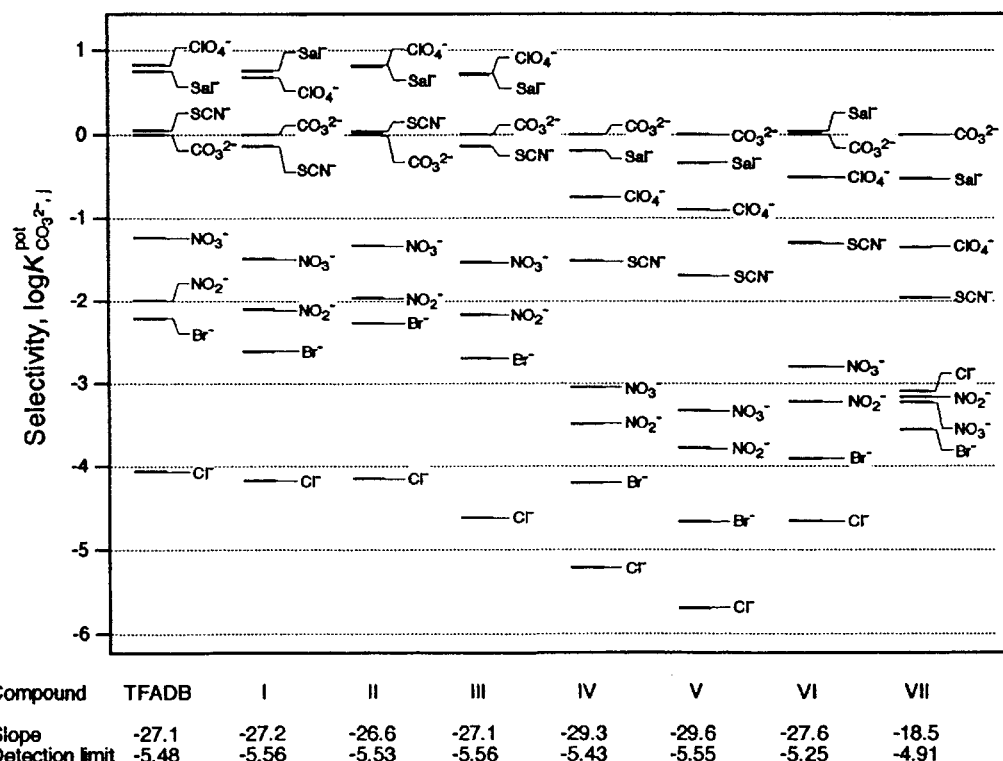
**Figure 2.** Carbonate selectivity, response slopes (mV/decade), and detection limits (logarithmic scale) of the electrodes based on cholic acid-based ionophores.
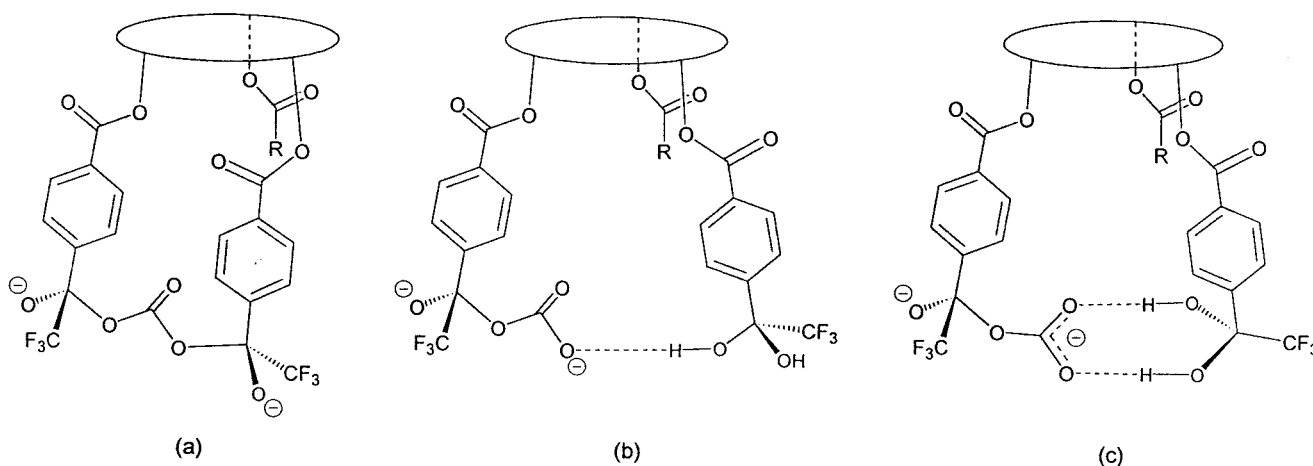
| Compound | TFADB | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|---|
| Slope | -27.1 | -27.2 | -26.6 | -27.1 | -29.3 | -29.6 | -27.6 | -18.5 |
| Detection limit | -5.48 | -5.56 | -5.53 | -5.56 | -5.43 | -5.55 | -5.25 | -4.91 |



(a)   (b)   (c)

**Figure 3.** Three possible structures for the adducts formed between carbonate and the cholic acid-based derivatives containing two trifluoroacetylbenzoyl groups [bis(TFAB)CA].

the equivalence of the C3, C7, or C12 position of the cholic acid. On the other hand, the electrodes based on **IV**−**VI** exhibited remarkably enhanced carbonate selectivity; in general, they provide 10−300-fold improved carbonate selectivity over various anions compared to that of the electrodes based on **I**−**III**. Especially noteworthy is their significantly reduced preference for the lipophilic anions, e.g., perchlorate, salicylate, and thiocyanate, resulting in the highest selectivity toward carbonate. This result supports our hypothesis that the two TFAP groups fixed on a rigid platform molecule indeed behave like molecular tweezers for the carbonate ion.

Of the three bis(TFAB)CA-based electrodes, that based on **V** [3,12-bis(TFAB)CA] exhibited the highest carbonate selectivity, followed by those based on **IV** [3,7-bis(TFAB)CA] and **VI** [7,12-

bis(TFAB)CA] in that order. Considering that the distances between the hydroxyl groups estimated with the AM1 semiempirical method are 4.9 Å for the C3−C7 pair, 4.6 Å for the C7−C12 pair, and 6.0 Å for the C3−C12 pair, while the optimal fitting distance between the carbonyl carbons of the two TFAB groups for carbonate is ∼4.8 Å, the trend observed does not coincide with our expectation. To explain the discrepancy, as illustrated in Figure 3, we assumed three different types of chemical bonding between the carbonate ion and the bis(TFAB)CAs. Carbonate ion may be bonded as follows: (a) covalently to the two TFAB groups; (b) covalently to one TFAB group and through hydrogen-bonding to another hydrated TFAB group; and (c) same as (b), but with two hydrogen bondings. However, the distances between the carbonyl carbons of two TFAB groups estimated at the AM1 level
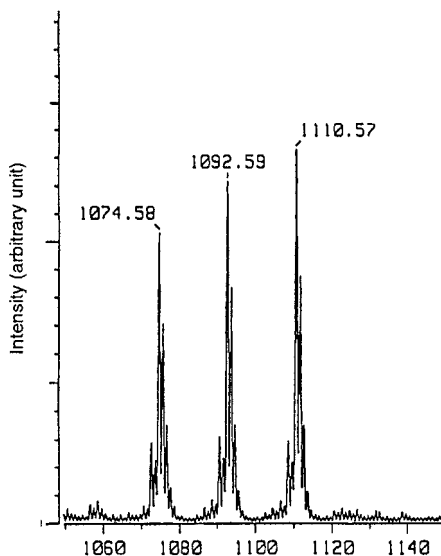
**Figure 4.** FAB MS of 3,12-bis(TFAB)CA (**V** in Figure 1). The first peak represents the parent compound and the second and third peaks represent the mono- and dihydrated compounds.



**Figure 5.** Calibration plots (activity scale) for the 3,12-bis(TFAB)-CA-based electrode to carbonate and various anions (salicylate, perchlorate, nitrate, chloride).



**Figure 6.** pH responses of the electrodes based on trifluoroacetyl-*p*-decylbenzene (TFADB), 3-(TFAB)CA (**I**), 3,12-bis(TFAB)CA (**V**), and 3,7,12-tris(TFAB)CA (**VII**).

semiempirical calculation, 7.4 Å for the C3−C7 pair (**IV**), 7.9 Å for the C3−C12 pair (**V**), and 7.3 Å for the C7−C12 pair (**VI**), are too far to covalently bond the carbonate ion without straining the structure. Hence, we supposed that the adducts formed between carbonate and bis(TFAB)CAs prefer the (b)- or (c)-type structure shown in Figure 3, which requires ∼1.6 Å extra distance than the (a)-type structure. The fast atom bombardment mass spectrum (FAB MS) in Figure 4 shows that significant fractions of **V**, and likewise those of **IV** and **VI**, are already hydrated with one or two water molecules before complexing the carbonate ion. We, thus, concluded that the monohydrated **V** could capture the carbonate ion with less structural strain. However, it does not explain the difference in carbonate selectivity among the three bis(TFAB)-CAs; more direct spectroscopic evidence is necessary to provide a reasonable explanation.

The electrode based on **VII** also exhibited higher carbonate selectivity over large anions, e.g., salicylate, perchlorate, and thiocyanate, but lower over small hydrophilic anions than those based on bis-TFAB compounds. Its potentiometric performance, the response slope (−18.5 mV/decade) and detection limit ($1.2 \times 10^{-5}$ M) for carbonate, was in general inferior to that of other electrodes based on **I**−**VI**. It was thought that the binding site formed by three pendant TFAB groups is too crowded for binding the carbonate ion.

Of the seven electrodes examined, that based on 3,12-bis-(TFAB)CA (**V**) resulted in the best potentiometric performance for detecting the carbonate ion: Figure 5 shows the calibration plots given in activity scale for carbonate and several selected anions. Considering that the therapeutic level of salicylate is ∼0.15−2.1 mM, while the TCO₂ level in normal serum is ∼25 mM, the calibration plots in Figure 5 suggest that the determination of TCO₂ in serum is possible by measuring the carbonate ion and pH simultaneously even in the presence of maximum level of salicylate interference. However, since the electrodes based on TFAP derivatives respond to carbonate only in alkaline pH conditions, the serum samples (pH ∼7.4) have to be diluted with a high-pH buffer before the measurements. For this reason, the
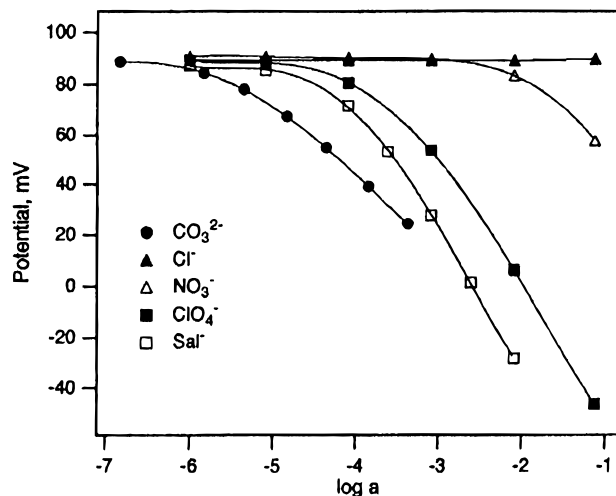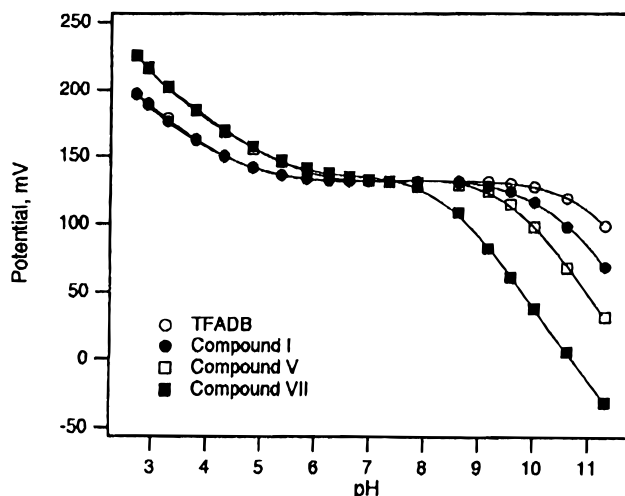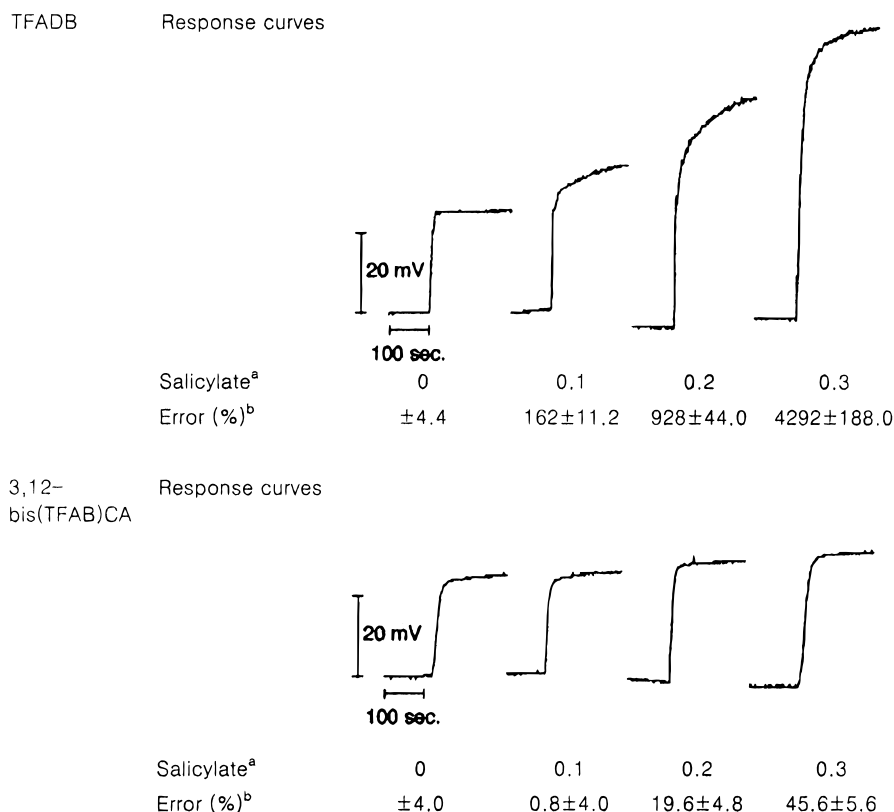
pH response of the 3,12-bis(TFAB)CA-based electrode is compared with those of the TFADB-, 3-(TFAB)CA-, and 3,7,12-tris-(TFAB)CA-based electrodes in Figure 6. It is seen that in the alkaline region the electrode based on 3,12-bis(TFAB)CA is more susceptible to hydroxide interference than those based on the compounds with a single TFAB group, but less than that based on three TFAB groups. It indicates that each TFAB group in 3,-12-bis(TFAB)CA additively increases its response to hydroxide interference. Thus, one needs to tightly control the pH of the sample solutions when the carbonate measurement is made with 3,12-bis(TFAB)CA or other TFAP derivative-based electrodes.

Dynamic step responses of the 3,12-bis(TFAB)CA-based electrode to 10 times-diluted serum samples containing 25 mM TCO₂ and 0−3 mM salicylate are shown in Chart 1 with relative error percent with respect to the known TCO₂ concentration. It is seen that the electrode based on 3,12-bis(TFAB)CA provides much enhanced analytical performance over that based on TFADB; the level of error caused by 0.1 mM salicylate is less than 5% and practically governed by the precision of the reading device. This result is comparable to that obtained with the extensively modified

**Chart 1. Potentiometric Responses of Carbonate-Selective Electrodes to 10 Times-Diluted Serum Samples Containing 25 mM TCO$_2$ and 0$-$3 mM Salicylate with 0.1 M Tris-H$_2$SO$_4$ (pH 8.6)**

TFADB          Response curves

|  20 mV  |  100 sec.  |  |  |  |
|---|---|---|---|---|
| Salicylate[a] | 0 | 0.1 | 0.2 | 0.3 |
| Error (%)[b] | ±4.4 | 162±11.2 | 928±44.0 | 4292±188.0 |

3,12–bis(TFAB)CA          Response curves

|  20 mV  |  100 sec.  |  |  |  |
|---|---|---|---|---|
| Salicylate[a] | 0 | 0.1 | 0.2 | 0.3 |
| Error (%)[b] | ±4.0 | 0.8±4.0 | 19.6±4.8 | 45.6±5.6 |

[a] Unit, mM. [b] Error with respect to 2.5 mM TCO$_2$.

asymmetric carbonate-selective membrane.[20] As we have demonstrated previously, slight modification of the membrane matrix, e.g., the type of polymer and the ratio of mobile cationic sites to the neutral carrier, should result in the carbonate-selective electrode system that is not subject to interference from lipophilic anions. Studies in that direction are in progress to develop the clinical and oceanic carbon dioxide measurement system.

## CONCLUSION

The carbonate-selective neutral carriers have been prepared by coupling one to three TFAB groups to a cholic acid derivative which has three hydroxyl linkers on the C3, C7, and C12 positions of its rigid steroidal ring structure. It was observed that the electrodes based on bis(TFAB)CA compounds provide the truly enhanced carbonate selectivity over most anions of clinical and environmental interests, indicating they behave like molecular tweezers for the carbonate ion. Since the distances between the carbonyl carbons of the two TFAB groups are calculated to be larger than 7.3 Å at the AM1 level semiempirical calculation, and significant fractions of bis(TFAB)CA compounds are mono- or dihydrated as evidenced by their FAB MS, it was supposed that the carbonate ion is bonded covalently to the carbonyl carbon of one TFAB group and hydrogen-bonded to the hydroxyl group of another hydrated TFAB. The analytical utility of 3,12-bis(TFAB)-CA has been demonstrated by measuring the carbon dioxide species in human serum in the presence of lipophilic anion, e.g., salicylate.