

Enhanced Serum Carbon Dioxide Measurements with a Silicone Rubber-Based Carbonate Ion-Selective Electrode and a High-pH Dilution Buffer

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A new silicone rubber matrix carbonate-selective membrane and a high-pH buffer diluent are used to enhance the performance of the electrode measurements for serum carbon dioxide. The proposed membrane employs one-component silicone rubber as the matrix and trifluoroacetyl-*p*-decylbenzene as the neutral ionophore. The optimized membrane formulation incorporates as high as 21.9 wt % plasticizer (e.g., bis(2-ethylhexyl) adipate). The highly plasticized silicone rubber membranes not only function equivalently, in terms of the carbonate response, to the conventional PVC matrix membranes, but they also exhibit substantially reduced interfering response toward salicylate. Furthermore, the silicone rubber membrane exhibits better adhesion to the solid surface than do PVC or PU membranes. The use of higher pH buffers (e.g., 2-amino-2-methyl-1-propanol (AMP)-H₂SO₄, pH 9.5–10.5) further enhances the selectivity of the carbonate electrode measurement system for total CO₂ species over other anions. It is shown that the combined use of the silicone rubber matrix membrane and the high-pH AMP buffer provides a carbonate sensor system that is substantially less subject to interference from salicylate and chloride than is the conventional measurement system employing the PVC-based electrode with the lower pH (8.4–8.8) buffer diluent.

Interest in using polymer membrane type carbonate ion-selective electrodes (ISEs) for total carbon dioxide measurements, rather than using CO₂ gas sensors with the Severinghaus configuration, arises from the rapid response and recovery times and ease of fabrication and miniaturization associated with these devices. Indeed, several commercial automated analyzers employ carbonate ISEs as flow-through detectors for such purposes.^{1–3} These carbonate-selective membranes usually consist of a trifluoroacetophenone derivative (e.g., trifluoroacetyl-*p*-butylbenzene or

p-decylbenzene; TFABB or TFADB) as a neutral ionophore, a polymer matrix, a plasticizer, and a lipophilic additive.^{3–8}

As previously reported, the conventional PVC-based carbonate-selective membranes doped with TFABB or TFADB exhibit a high selectivity for salicylate.^{3,5–7} When these membrane electrodes are used for human serum samples, salicylate can interfere with carbon dioxide measurements. Indeed, several reports indicate that when automated clinical analyzers employing the carbonate ISEs are used, salicylate produces a positive interference for carbon dioxide measurements in serum samples.^{1–3} Several approaches to minimize such salicylate interference problems have been reported previously. For instance, Scott et al.³ employed an anion-binding complexone such as mercuric EDTA in the buffer reagent to decrease salicylate interference on the carbonate-responsive membranes. More recently, we⁹ reported the use of asymmetric cellulose triacetate type ISEs to overcome the salicylate interference problems in serum carbon dioxide measurements.

In this report, we describe the development of a new silicone rubber matrix carbonate-selective membrane that exhibits significantly reduced response toward salicylate and the use of a new buffer reagent that further minimizes salicylate interference by virtually enhancing the CO₂ selectivity of the carbonate sensor system. Recently, one-part vulcanizing silicone rubber has received attention as a possible alternative to PVC for fabricating solid-state type ion sensors because it exhibits a high bond strength to a wide range of substrates.^{10–13} Unlike PVC matrix

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membranes, however, the application of such silicone rubber membranes has been successful only for several cation-sensing systems (i.e., K^+ /valinomycin, NH_4^+ /nonactin, and H^+ /tridodecylamine).^{10–13} Furthermore, no report has yet described the use of silicone rubber materials as the matrix of trifluoroacetophenone type carbonate-selective membranes, to the best of our knowledge. However, the silicone rubber membranes optimized in the present study not only function equivalently, in terms of the carbonate response, to the conventional PVC matrix membranes, but they also exhibit substantially reduced interfering response toward salicylate. Furthermore, the silicone rubber membrane exhibits better adhesion to the solid surface than do PVC membranes.

It is necessary to perform electrode measurements in a buffered solution of a relatively high pH when the carbonate sensor system is used for the determination of total CO_2 . For this, tris(hydroxymethyl)aminomethane, buffered with HCl or H_2SO_4 to a pH range of 8.4–8.8,^{1–5,9} has been employed as the buffer reagent almost without exception. This is because the TFABB- or TFADB-based electrodes are known to exhibit a large response to hydroxide ion at high pH,^{5,8} and a working pH higher than 8.75 would result in poorer detection limits toward total CO_2 .⁵ However, we found that the use of higher pH buffers (e.g., 2-amino-2-methyl-1-propanol (AMP)- H_2SO_4 , pH 9.5–10.5) enhances the selectivity of the carbonate electrode measurement system for total CO_2 species over other anions. In this work, we demonstrate that the combined use of the silicone rubber matrix membrane and the high-pH AMP buffer provides a carbonate sensor system that is substantially less subject to interference from salicylate and chloride than are similar systems reported so far.

EXPERIMENTAL SECTION

Reagents. Poly(vinyl chloride) (PVC), tridodecylmethylammonium chloride (TDMACl), and bis(2-ethylhexyl) adipate (DOA) were purchased from Fluka (Ronkonkoma, NY), while tris(hydroxymethyl)aminomethane (Tris) and 2-amino-2-methyl-1-propanol (AMP) were from Sigma (St. Louis, MO). Trifluoroacetyl-*p*-decylbenzene (TFADB; *p*-decyl- α,α,α -trifluoroacetophenone) was obtained from Trans World Chemicals (Rockville, MD), Tecoflex polyurethane (PU; SG-80A) from Thermedics (Woburn, MA), and one-component silicone rubber (3140 RTV) from Dow Corning (Midland, MI).

Preparation of Polymer Membranes. PVC, PU, or silicone rubber matrix membranes were prepared according to the method of Simon and co-workers.¹⁴ The composition of PVC- or PU-based carbonate-selective membrane was 4.9 wt % TFADB, 1.2 wt % TDMACl, 39.1 wt % PVC or PU, and 54.8 wt % DOA. Silicone rubber membranes were prepared by dissolving appropriate amounts of 3140 RTV, TFADB, TDMACl, and DOA (when needed) in THF and casting the mixture in a glass ring placed on a flat Teflon plate. Table 1 summarizes the compositions of the carbonate-selective polymer membranes examined in this study.

Evaluating Potentiometric Response and Selectivity. Electrochemical properties of the carbonate-selective membranes were evaluated in the conventional ISE configuration as described

Table 1. Compositions of the Polymer Matrix Carbonate-Selective Membranes Studied^a

membrane no.	PVC	PU	silicone rubber	TFADB ^b	TDMACl ^c	DOA ^d
1	39.1			4.9	1.2	54.8
2		39.1		4.9	1.2	54.8
3			93.9–83.3	4.9	1.2–11.8	
4			80.6	4.2	1.0	14.2
5			63.5	9.7	4.9	21.9

^a In wt %. ^b Trifluoroacetyl-*p*-decylbenzene. ^c Tridodecylmethylammonium chloride. ^d Bis(2-ethylhexyl) adipate.

previously.^{9,12} Selectivity coefficients were determined by using the matched potential method¹⁵ at an interfering ion concentration of 0.1 M. The detection limits of the membrane electrodes were obtained from the calibration curves as described elsewhere.¹⁶

For a preliminary solid-state sensor application, a coated wire electrode (CWE) was fabricated according to a modification of the method previously described by Cattrall and Freiser.¹⁷ A silver wire (2-cm length; 1-mm i.d.), soldered to the central conductor of coaxial cable wire, was used as the metallic substrate. The silver wire was fitted into a glass tube (1.3-mm i.d.), with the tip of the wire exposed. The tip of the wire was coated by dipping about 5 mm of the wire assembly several times into the silicone rubber coating solution (membrane 5 composition).

Evaluating Membrane Adhesion. The Scotch tape test¹⁸ and the ultrasonic bath test¹⁹ were employed to estimate the adhesion of the membranes to the glass surface. The spots (10–20- μ m thickness; 2-mm i.d.) of each membrane formulation (refer to Table 1) were formed on a cleaned glass plate with a 250- μ L microsyringe under a microscope. After curing, the number of spots removed by the adhesion tests as recorded. In the case of the ultrasonic bath test, a 2-h vibration period was employed.

RESULTS AND DISCUSSION

Typically, silicone rubber matrix ion-selective membranes do not employ plasticizer.^{10–13} The high electrical resistance of the silicone rubber membranes is usually decreased upon incorporation of the ionophore to the matrices. In some cases, the signal noise associated with high resistance can be further decreased by adding lipophilic ionic additives (e.g., potassium tetrakis(*p*-chlorophenyl)borate in the case of cation-selective membranes).^{12,13} However, we found that such an approach (i.e., the addition of cationic additives in this case) is not successful for the trifluoroacetophenone-based silicone rubber matrix system. For instance, TFADB-doped silicone rubber membranes employing TDMACl (up to 12 wt %) as an additive showed negligible ion responses with a high signal noise (refer to the composition of membrane 3 in Table 1). However, such silicone rubber matrix membranes, when plasticized (~14.2–37.2 wt %), exhibited electrochemical performance comparable to those of the conventional PVC or PU matrix membranes. As can be seen from Figure 1, the carbonate

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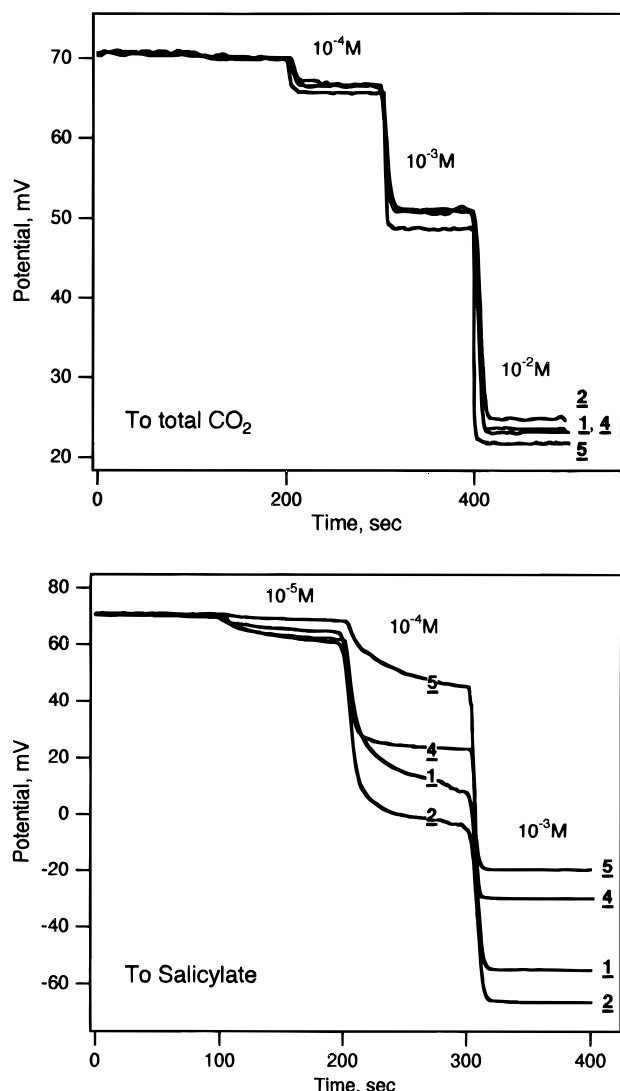


Figure 1. Dynamic response of TFADB-based membranes prepared by employing different membrane compositions: (1) PVC, (2) polyurethane, (4) silicone rubber with 14.2 wt % plasticizer, and (5) silicone rubber with 21.9 wt % plasticizer (refer to Table 1 for the detailed compositions). The background electrolyte was 0.2 M Tris- H_2SO_4 , pH 8.6.

response of the TFADB-doped membrane employing 14.2 wt % plasticizer (membrane 4) was virtually the same as that of the PVC (membrane 1) or PU (membrane 2) matrix membrane: these three membranes contain the same amount of TFADB (8.6 mg) and TDMACI (2 mg). It can be seen, however, that the plasticized silicone rubber membrane exhibits the least response toward salicylate and the PU membrane the greatest. We have optimized the performance of the silicone rubber matrix membrane by varying the ratio of the membrane components employed. The final optimized membrane consists of 63.5 wt % silicone rubber, 9.7 wt % TFADB, 4.9 wt % TDMACI, and 21.9 wt % DOA (i.e., membrane 5 in Table 1). As can be seen in Figure 1, this highly plasticized silicone rubber matrix membrane exhibits a further decreased interfering response toward salicylate with an enhanced response toward carbonate. When compared to the conventional PVC-based membrane, the new silicone rubber membrane exhibited enhanced selectivities over most other anionic species (measured at pH 8.6) except OH^- : $\log k^{\text{pot}}_{\text{CO}_2/\text{j}}$ ($\text{j} = \text{Cl}^-, \text{NO}_3^-, \text{SCN}^-, \text{salicylate}, \text{and OH}^-$) = -2.8, 0.5, 1.9, 2.6, and 0.7 for PVC

(membrane 1); -4.0, -0.8, 0.9, 2.1, and 1.1 for silicone rubber (membrane 5).

Strongly adhesive characteristics of silicone rubber-based ion-selective membranes, prepared without addition of plasticizer, have been demonstrated previously by Goldberg et al.^{12,13} In this preliminary study, we compared the adhesion of the membrane formulations (listed in Table 1) to the glass surface by employing the simple tape¹⁸ and ultrasonic bath¹⁹ tests, as described in the Experimental Section. Both tests, though yielding not very quantitative results, indicate that addition of plasticizer to the silicone rubber matrix does not significantly deteriorate its adhesive property, and even a highly plasticized silicone rubber membrane retains adhesion much stronger than that of the PVC or PU matrix membrane: in both tests, all of the PVC films were removed from the glass substrate, while none of the silicone rubber films (membranes 3, 4, and 5) came off. In the case of the PU matrix (membrane 2), none of the spots pulled off the substrate in the tape test, while all of the spots were detached in the bath test.

In an aqueous solution, CO_2 species exist in various forms (i.e., CO_2 , H_2CO_3 , HCO_3^- , CO_3^{2-} , etc.). The fraction of each species present in the solution is dependent on the pH of the solution. Therefore, the pH of the sample needs to be fixed by using an appropriate buffer to determine the total CO_2 content with a carbonate ISE. Increasing the pH will increase the fraction of the carbonate species in the sample solution, thereby increasing the carbonate response signal of the electrode. However, it has been shown that the trifluoroacetophenone type electrodes also respond to hydroxide ion (OH^-), and thus, at high pHs (>9), OH^- ions interfere with the CO_2 measurements when such electrodes are utilized.^{5,8} A pH range of 8.4–8.8 has been found to be an optimal buffer range (i.e., a trade-off pH range) and has been used for almost all carbonate ISEs employing the trifluoroacetophenone derivatives, including TFADB.^{1–5,9}

However, it should be noted that the working pH of such a carbonate electrode measurement system has been optimized with respect to the slope and detection limit of the electrode for CO_2 species. Apparently, the fact that the trifluoroacetophenone type electrodes exhibit a large interfering response toward salicylate has not been considered. This is important, particularly when such carbonate electrodes are used for human serum samples: salicylate can produce a positive interference for the samples from the patients taking aspirin. In this regard, we have examined the feasibility of using a higher pH buffer (pH >9.0) to enhance the practical selectivity of the carbonate measurement system for total CO_2 species over salicylate. Since the pK_a values of salicylic acid are 2.97 (pK_1) and 12.38 (pK_2), it exists mainly as an anionic form at alkaline pH (e.g., at pH 11, 96% of the total species is present as the monovalent anionic form (i.e., salicylate) and 4% as the dianion). Thus, further increasing the pH will have only a negligible effect on the fraction of salicylate species while significantly increasing the amount of carbonate species in the solution at this high-pH region (e.g., pH 8–11).

For the carbonate electrode measurements, the most widely used buffer reagent has been Tris, buffered with HCl or H_2SO_4 (Tris-HCl or Tris- H_2SO_4), which has a useful pH range of 7.0–9.0. We have examined a wide range of buffer reagents that can produce a useful pH higher than 9: AMP, monoethanolamine, piperazine, triethylamine, cyclohexylamine, CHES, CAPS, etc. We have found that the carbonate electrode exhibits an interfering

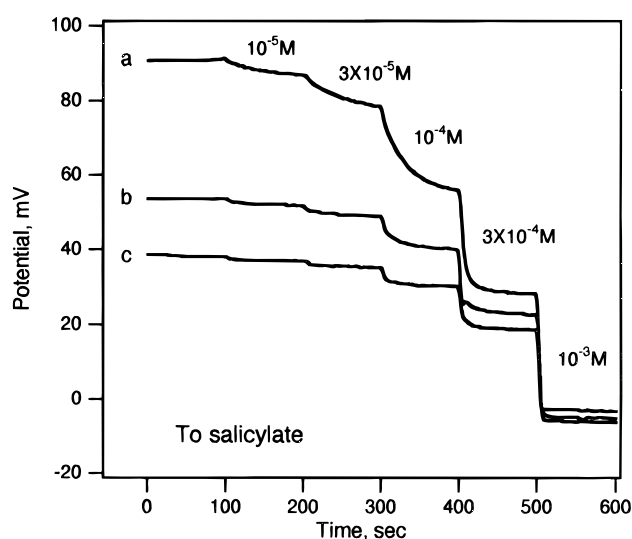
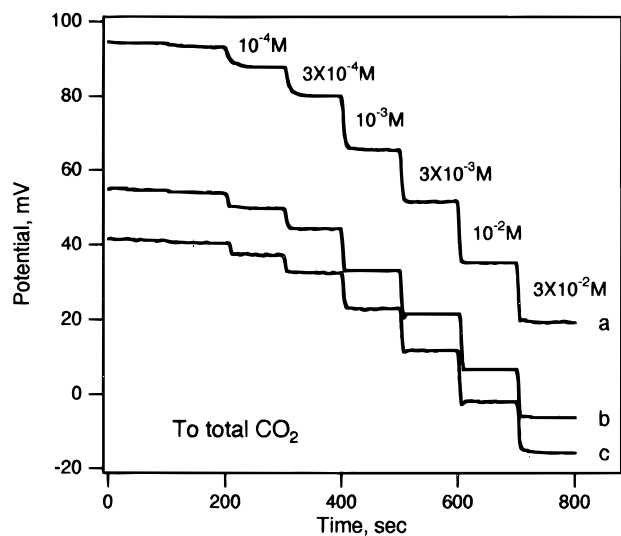


Figure 2. Dynamic response of the silicone rubber (membrane 5) electrode toward CO_2 and salicylate species in three different background electrolytes: (a) 0.2 M Tris- H_2SO_4 , pH 8.6; (b) 0.2 M AMP- H_2SO_4 , pH 9.7; and (c) 0.2 M AMP- H_2SO_4 , pH 10.4.

emf signal in the background electrolyte made with acid-type buffer reagents. It was thought that the TFADB-based membrane responds, to a significant degree, toward a negatively charged buffering species. Among those tested, we found that two base-type reagents, AMP and monoethanolamine, offer satisfactory electrode responses, and we chose AMP for the subsequent experiments.

Figure 2 compares the dynamic responses of the plasticized silicone rubber electrode (membrane 5) toward carbonate and salicylate species in three different buffer systems: (a) 0.2 M Tris- H_2SO_4 , pH 8.6; (b) 0.2 M AMP- H_2SO_4 , pH 9.7; and (c) 0.2 M AMP- H_2SO_4 , pH 10.4. As the pH of the buffer increased, the emf signal of the electrode in the background electrolyte shifted to the more negative potential due to the hydroxide response of the electrode. As can be seen, the electrode showed a similar emf reading toward relatively high salicylate concentrations ($\sim 10^{-3}$ M) in all three different pH buffer solutions. However, it can be clearly seen that, for the same amount of total CO_2 , the electrode exhibits a greater emf signal in a higher pH buffer, due to an increased fraction of carbonate species present in the solution. As expected, the carbonate slope was decreased as the pH of the buffer increased:

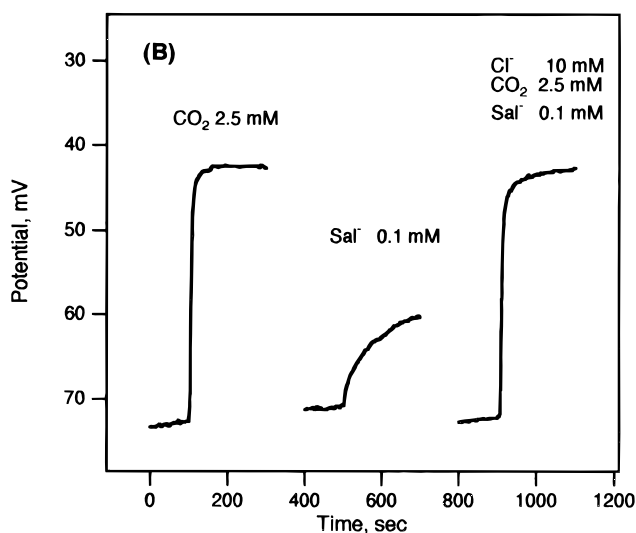
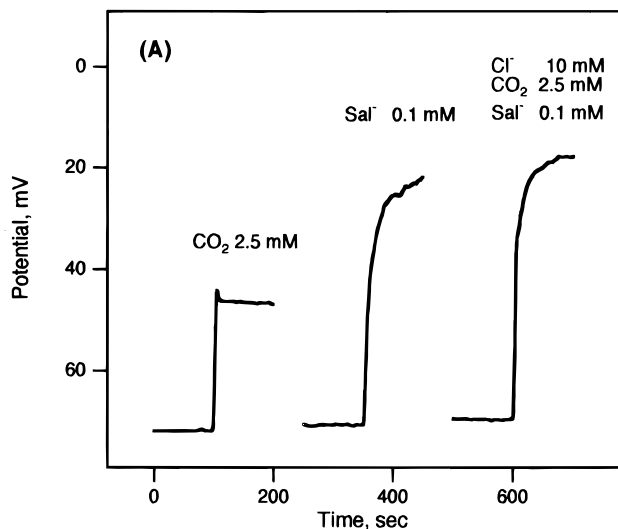


Figure 3. Response comparison of the conventional and the new CO_2 measurement systems: (A) PVC membrane 1 with 0.2 M Tris- H_2SO_4 , pH 8.6, and (B) silicone rubber membrane 5 with 0.2 M AMP- H_2SO_4 , pH 9.7.

slope (mV/decade; in the range of 0.3–30 mM total CO_2) = -30 for pH 8.6; -26 for pH 9.7; and -24 for pH 10.4. However, the detection limit of the electrode was not significantly changed as the pH of the buffer increased: 0.11 mM for pH 8.6; 0.13 mM for pH 9.7; and 0.16 mM for pH 10.4. Most importantly, the practical selectivity of the electrode for total CO_2 over salicylate was improved as the pH of the buffer increased: $\log k^{\text{pot}}_{\text{CO}_2, \text{sal}^-} = 2.1$ for pH 8.6; 1.5 for pH 9.7; and 1.1 for pH 10.4. The theoretically expected selectivity improvement due to the pH change is 3.3 times (from pH 8.6 to 9.7) or 5.5 times (from pH 8.6 to 10.4), calculated from the known equilibrium constants by assuming a Nernstian response of the electrode. This type of selectivity enhancement is also expected over other anions, such as chloride, that exist mainly as an anionic form in the solution.

Figure 3 further demonstrates the enhanced performance and the analytical utility of the new measurement system (i.e., the combined use of the new silicone rubber membrane and the high-pH diluent): (A) PVC membrane 1 with Tris- H_2SO_4 , pH 8.6, and (B) silicone rubber membrane 5 with AMP- H_2SO_4 , pH 9.7. In this preliminary experiment, we compared the response properties of the electrodes toward 2.5 mM CO_2 , 0.1 mM salicylate, or a

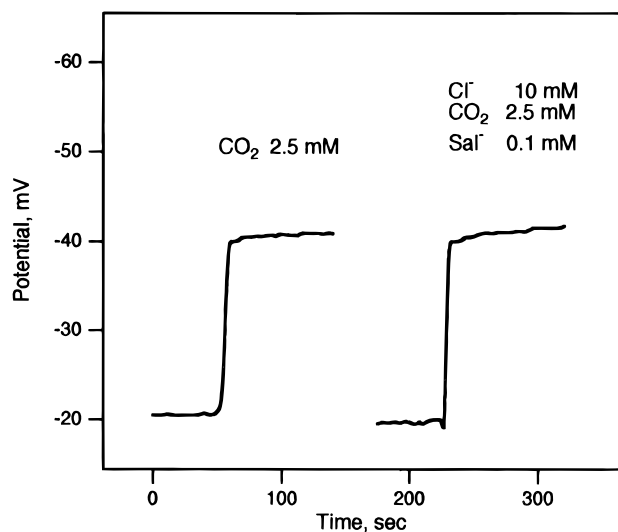


Figure 4. Response properties of the CWE type solid-state sensor prepared with the silicone rubber matrix (membrane 5). The background electrolyte was 0.2 M AMP- H_2SO_4 , pH 9.7.

mixture containing 2.5 mM CO_2 , 0.1 mM salicylate, and 10 mM chloride (i.e., to mimic CO_2 measurements for serum samples diluted 10 times with buffer; the normal levels of serum CO_2 and chloride are 21–31 and 101–111 mM, respectively and the therapeutic level of salicylate is 0.15–2.1 mM). The slope of the new CO_2 measurement system was determined to be -25 mV/decade in the range of 1–10 mM total CO_2 . As can be seen in Figure 3, the conventional PVC/Tris-based measurement system exhibited a severe interference from salicylate at a level comparable to the therapeutic level in the serum matrix. However, when the new silicone rubber/AMP-based system was employed, the presence of salicylate interference did not significantly alter the observed potentiometric response to 2.5 mM total CO_2 .

A similar experiment was done with a coated wire type electrode to examine the feasibility of adapting the new system

to the solid-state sensor formats. Such devices do not require an internal reference solution between the ion-selective polymer membrane and the inner metallic electrode and thus offer the advantages of miniaturization and mass fabrication for cost reduction. Figure 4 illustrates the dynamic response of the coated wire electrode employing the plasticized silicone rubber (membrane 5) toward 2.5 mM CO_2 and a mixture of 2.5 mM CO_2 , 0.1 mM salicylate, and 10 mM chloride in 0.2 M AMP- H_2SO_4 , pH 9.7. As can be seen, the coated wire electrode performed very well in detecting carbonate species and decreased the salicylate response in a fashion similar to the membrane mounted in the conventional electrode bodies.

In summary, we have described two different findings in this paper: the silicone rubber matrix carbonate-selective membrane and the new buffer diluent for the carbonate measurement system. The combined use of the highly plasticized silicone rubber electrode and the high-pH AMP buffer is shown to exhibit a substantially reduced response to salicylate, compared to the conventional PVC/Tris buffer-based system. Work on further demonstrating the clinical utility of the proposed system (i.e., real sample studies) is currently in progress, as are efforts to adapt the plasticized silicone rubber matrix to other ion-selective membrane systems.

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