

Asymmetric Carbonate Ion-Selective Cellulose Acetate Membrane Electrodes with Reduced Salicylate Interference

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The asymmetric carbonate ion-selective cellulose triacetate membrane electrodes are used to reduce salicylate interference for total CO₂ measurements in human serum samples. The asymmetric membranes are formed by first casting a thin layer of cellulose triacetate without carrier, hydrolyzing one side of this thin film with base, and, subsequently, casting a second layer of cellulose triacetate containing the membrane active components [i.e., (trifluoroacetyl)-*p*-butylbenzene as a neutral ionophore]. The resulting asymmetric carbonate-selective membranes function equivalently, in terms of the carbonate response, to conventional PVC-based membranes. However, asymmetric membranes exhibited a reduced and slower response toward large lipophilic anions such as perchlorate or salicylate compared to PVC-based membranes, while both membranes showed similar response characteristics toward smaller hydrophilic anions such as carbonate. The asymmetric carbonate ion-selective membranes can be used to enhance the selectivity for carbonate ion over other large anions by employing either the flow-through or the equilibrium (static) arrangements. For preliminary applications, the asymmetric carbonate ion-selective membranes were utilized to determine total CO₂ contents in flowing or static solutions containing salicylate interferences. It is shown that the asymmetric membranes are substantially less subject to salicylate interference compared to the conventional PVC-based membranes.

INTRODUCTION

The accurate and rapid determination of total carbon dioxide species in physiological, industrial, and environmental samples presents a formidable challenge. Automated clinical analyzers are now routinely used for the determination of total carbon dioxide in biological fluids. Several commercial automated analyzers (Technicon RA-1000, Kodak Ektachem 400, etc.) employ carbonate-selective electrodes as flow-through detectors for such purposes.¹⁻³ However, these

devices show severe interference from several sources, particularly from salicylate.

Previously described carbonate ion-selective membranes usually consist of trifluoroacetophenone derivatives [e.g., (trifluoroacetyl)-*p*-butylbenzene; TFABB] as neutral ionophores, a polymer matrix [e.g., poly(vinyl chloride); PVC], a plasticizer [e.g., bis(2-ethylhexyl) sebacate; DOS], and lipophilic additives [e.g., tridodecylmethylammonium chloride; (TDMA)Cl].³⁻⁷ PVC is by far the most commonly used polymer matrix for such membrane electrodes.

As previously reported, TFABB-based carbonate-selective PVC membranes exhibit much higher selectivity for salicylate over carbonate ion.^{3,5-7} When such carbonate-selective membrane electrodes are used for physiological samples (e.g., human serum), salicylate can interfere with carbon dioxide measurements. Salicylate and its analogues (e.g., aspirin) are commonly used as effective analgesics, and recommended therapeutic levels in serum range from 0.15 to 2.1 mmol/L.^{8,9} The normal level of total carbon dioxide for human serum is 21–31 mmol/L.¹⁰ Indeed, several reports indicate that when automated clinical analyzers using carbonate ion-selective membranes are used, salicylate produces a positive interference for carbon dioxide measurements in serum samples.¹⁻³

Several approaches have been reported in order to minimize problems with salicylate interference. For instance, Scott et al. reported on the use of an anion-binding complex such as mercuric EDTA in the buffer reagent to decrease salicylate interference on carbonate-responsive membranes.³ The RA-1000 system employs a software program in order to detect any salicylate interference and print an error code next to the carbon dioxide results.²

In this report a new approach is described to minimize such salicylate interference problems. Asymmetric membrane technology is utilized to enhance the performance of the resulting membrane electrodes, in terms of potentiometric

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Table I. Comparison of the Static Mode Potentiometric Selectivity Coefficients for TFABB-Doped Unmodified CTA (n-CTA) and Asymmetric CTA Membrane Electrodes^a

matrix	Cl ⁻	pyruvate	oxalacetate	NO ₃ ⁻	I ⁻	ClO ₄ ⁻	salicylate
n-CTA	-2.54	-0.48	-0.49	-0.33	0.43	1.93	2.04
asym-CTA	-2.52	-1.54	-1.54	-0.36	0.43	1.76	1.72

^a Evaluated in 0.1 mol/L Tris-H₂SO₄, pH 8.6. The separate solution method was used by employing concentrations instead of activities. The interfering anion concentration was 0.01 mol/L except chloride (0.1 mol/L) and salicylate (3 × 10⁻⁴ mol/L).

ion selectivities. The use of asymmetric cellulose triacetate membranes in biosensor applications has been previously described.^{11,12} In previous work, asymmetric ion-selective membranes were utilized to devise methods for covalent attachment of bioreagents directly to the surface of polymeric ion-selective membrane electrodes, by making use of the hydroxyl functional groups on the hydrophilic side of the asymmetric membranes.

In this work, however, the asymmetric carbonate ion-selective membrane electrodes are utilized as the flow-through detector to determine total carbon dioxide content in flowing solutions containing salicylate interference. The practical analytical application of the proposed asymmetric membrane electrodes is demonstrated by measuring the carbon dioxide contents in human blood serum.

EXPERIMENTAL SECTION

Reagents. Poly(vinyl chloride) (PVC), cellulose triacetate (CTA), bis(2-ethylhexyl) sebacate (DOS), and bis(2-ethylhexyl) adipate (DOA) were purchased from Fluka (Ronkonkoma, NY). (Trifluoroacetyl)-*p*-butylbenzene (TFABB) was obtained from Specialty Organics, Inc. (Irwindale, CA), tridodecylmethylammonium chloride [(TDMA)Cl] from Polysciences, Inc. (Warrington, PA), and salicylic acid, sodium salt, from Aldrich Chemical Co. (Milwaukee, WI).

All other chemicals used were analytical-reagent grade. Standard solutions and buffers were prepared with deionized water.

Preparation of Polymer Membranes. PVC- or unmodified CTA-matrix carbonate-selective membranes were prepared by the method of Simon and co-workers.¹³ The composition was usually 9.5 wt % TFABB, 37.5 wt % polymer (PVC or CTA), 52 wt % plasticizer (DOS or DOA), and 1.0 wt % (TDMA)Cl. Asymmetric CTA carbonate-selective membranes were prepared according to the method of Cha and Meyerhoff.¹¹

Evaluating Potentiometric Response and Selectivity. Electrochemical properties of the membranes were evaluated in the conventional ion-selective electrode configuration. For all types of membranes, after curing, 5.5-mm-diameter disks were cut out and placed in Phillips electrode bodies (IS-561) (Glasblaserei Moller, Zurich, Switzerland). In the case of the asymmetric CTA membranes, disks were mounted in electrode bodies with the hydrolyzed side facing the outer sample solution. For all carbonate ion-selective electrodes, an aqueous mixture of 0.1 mol/L NaH₂PO₄, 0.1 mol/L Na₂HPO₄, and 0.01 mol/L NaCl was employed as the internal filling solution. The external reference electrode was an Orion sleeve-type double-junction Ag/AgCl electrode (Model 90-02). Electrodes were connected through a high-impedance amplifier to an IBM AT-type computer equipped with an analog-to-digital converter or through a Fisher Accumet Model 157 electrode switch box to a Fisher Accumet Model 620 pH-mV meter (Romulus, MI).

Static Mode Potentiometric Measurements. Initial potentiometric evaluation of various matrix membranes was conducted by employing a static arrangement. The calibration plots were obtained from addition of standard solutions to 200 mL of background electrolyte (0.1 mol/L Tris-H₂SO₄, pH 8.6) at room

temperature. The solutions were magnetically stirred throughout, and the equilibrium potentials were recorded. Selectivity coefficients were determined by the separate solution method.¹⁴

Flow-Injection Measurements. All measurements were made in the wall-jet configuration. A Phillips electrode body was fitted with a special flow cap for use as a flow-through detector in a large volume wall-jet cell.¹⁵ An Orion Ag/AgCl reference electrode along with the working carbonate ion-selective electrode was placed in a large beaker of reagent buffer (0.1 mol/L Tris-H₂SO₄, pH 8.6). Teflon tubing (0.012-in. i.d.) connected the flow cap to the injection valve. A peristaltic pump (Ismatech Model 7331-00, Zurich, Switzerland) and a Rheodyne four-way rotary valve (Model 7125) equipped with a sample loop completed the flow-injection setup. For these studies, 150 µL of various anion standard or mixture solutions was injected into a buffer stream (7.5 mL/min) of 0.1 mol/L Tris-H₂SO₄, pH 8.6. The standards were prepared with the same buffer solution. The emf values were recorded every 0.1 s at room temperature, using the high-impedance amplifier/analog-to-digital converter setup described in the previous section.

Measurements of Carbonate in Serum Samples. In order to study the effect of serum matrix on the carbonate response of the TFABB-based PVC and asymmetric CTA membranes, SeraChem Level 1 Clinical Chemistry Control Human Serum-Assayed (Fisher Scientific, Orangeburg, NY) was used. For these studies, the potentiometric measurements were made in a static mode by adding 2 mL of the reconstituted serum samples to 18 mL of background electrolyte (0.1 mol/L Tris-H₂SO₄, pH 8.6). For the interference study, the required amount of NaHCO₃ salt was added to a mixed reconstituted control serum samples (from five 5-mL bottles) to make a final total carbon dioxide concentration of 25 mmol/L. Portions (5 mL) of this serum sample were then spiked with varying amounts of salicylate by adding 50-µL aliquots of salicylate standard solutions. The response of the PVC- and asymmetric CTA-based membranes toward the serum samples was compared by using the high-impedance amplifier/analog-to-digital converter setup. The emf values were recorded every 1 s at room temperature, and the membrane electrodes were washed with deionized water between measurements. These results were duplicated, and the data were reproducible.

RESULTS AND DISCUSSION

The hydrophilic layer of the asymmetric membrane is formed directly from one side of the nonplasticized mother cellulose triacetate layer by base hydrolysis, and thus an integral part of the modified CTA membrane.¹¹ The ion-selective layer incorporating the membrane active components [TFABB, (TDMA)Cl, plasticizer] is then fused to the opposite side of the hydrophilic surface of the modified CTA membrane. Therefore, a strong adhesive strength exists between the hydrophilic layer and the ion-selective layer. The hydrophilic porous layer is immiscible with the hydrophobic organic plasticizer in which TFABB is dissolved and, thus, does not function as an ion-selective surface. However, when immersed in an aqueous solution, the hydrophilic layer of the

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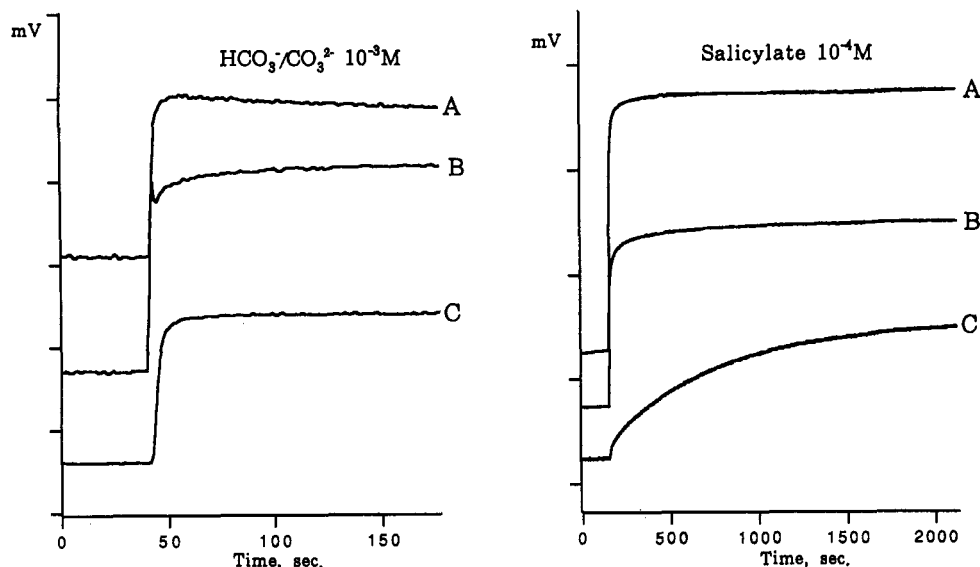


Figure 1. Typical dynamic response of carbonate ion-selective electrodes prepared with PVC (A), unmodified CTA (B), and asymmetric CTA (C) membranes (concentration change: from 10^{-4} to 10^{-3} mol/L for CO_2 ; from 10^{-5} to 10^{-4} mol/L for salicylate).

Table II. Static Mode Potentiometric Selectivity Coefficients Observed at Various Salicylate Concentrations for Unmodified CTA (*n*-CTA) and Asymmetric CTA Membrane Electrodes^a

matrix	$\log k_{\text{CO}_3, \text{salicylate}}^{\text{pot}}$ for interfering salicylate concn, mol/L						
	1×10^{-5}	3×10^{-5}	1×10^{-4}	3×10^{-4}	1×10^{-3}	3×10^{-3}	1×10^{-2}
<i>n</i> -CTA	0.57	0.97	1.56	2.04	2.71	3.18	3.73
asym-CTA	0.18	0.30	0.58	1.72	2.43	3.00	3.58

^a Evaluated in 0.1 mol/L Tris- H_2SO_4 , pH 8.6. The separate solution-matched potential method was used by employing concentrations instead of activities.

asymmetric membrane stays on the ion-selective layer indefinitely (experimentally more than several months).

Table I compares the response properties of the asymmetric and nonasymmetric (unmodified) carbonate-selective CTA-matrix membranes. As can be seen, asymmetric modification had little or no effect on the response of the membrane to small anions (e.g., carbonate, chloride, acetate, bromide, etc.). However, the asymmetric membrane exhibited reduced responses toward larger anions (e.g., salicylate and perchlorate), compared with the unmodified CTA-matrix membrane. Furthermore, the response of the asymmetric membrane electrodes to these large anions was extremely slow. The selectivity coefficients (Table I) reported in this study were calculated by using equilibrium response data taken 10 min after addition of the standard. Upon the standard addition of the large anions (e.g., salicylate or perchlorate), the asymmetric membrane electrodes did not reach equilibrium potentials even after this 10-min measurement period.

Figure 1 illustrates the dynamic response of the PVC-, CTA-, and asymmetric CTA-based carbonate-selective electrodes. All three membrane electrodes showed a similarly fast response upon additions of carbonate ion to the sample solution (concentration change: from 10^{-4} to 10^{-3} mol/L). However, while the PVC- and CTA-matrix membranes responded relatively quickly to a salicylate concentration change, the asymmetric membrane exhibited an extremely slow response when the salicylate concentration had been changed (from 10^{-5} to 10^{-4} mol/L). It should be noted that the time scales (*x*-axis) for two graphs in Figure 1 are significantly different. As can be seen, the response characteristics of the nonasymmetric CTA membrane are not very different from those of the PVC-matrix membrane. Both membranes exhibited similar dynamic response toward both carbonate and salicylate ions. From this fact, it was believed that the slow response kinetics of the asymmetric membrane

toward the large, hydrophobic anions is due to the presence of the additional hydrophilic layer on the surface of the ion-selective membrane.

As described previously, the ion-selective surface of the asymmetric membrane is covered with a thin hydrophilic layer. This hydrophilic layer acts like a cellulose dialysis membrane, the molecular cutoff of which is relatively small. In order for the asymmetric membrane to respond to anions when immersed in an aqueous solution, anions have to diffuse through the thin modified layer to the very surface of the inner ion-sensing layer. Because of the small pore size of the base-treated layer, however, large anions can pass through the layer only at a slow rate. In addition, the hydrophilicity of the layer retards, to a certain degree, the partitioning of hydrophobic anions such as salicylate or perchlorate through the hydrophilic layer. On the other hand, smaller, hydrophilic anions such as carbonate or chloride can easily permeate through this dialysis layer to the ion-sensing surface. Thus, the asymmetric membrane exhibits a reduced and slower response to a large, hydrophobic anions such as salicylate compared to the PVC- or the unmodified CTA-matrix membranes (i.e., kinetic/exclusion mechanism).

Table II indicates the selectivity coefficients of both the CTA and asymmetric CTA membranes, observed at various salicylate concentrations, and calculated by using the separate solution-matched potential method.¹⁶ As can be seen, at lower salicylate concentrations (less than 1 mmol/L), the asymmetric membrane achieved a greater degree of salicylate response reduction relative to the unmodified CTA-matrix membrane. At higher concentrations, however, the asymmetric membranes achieved only a small degree of response reduction. It is assumed that this behavior is observed because

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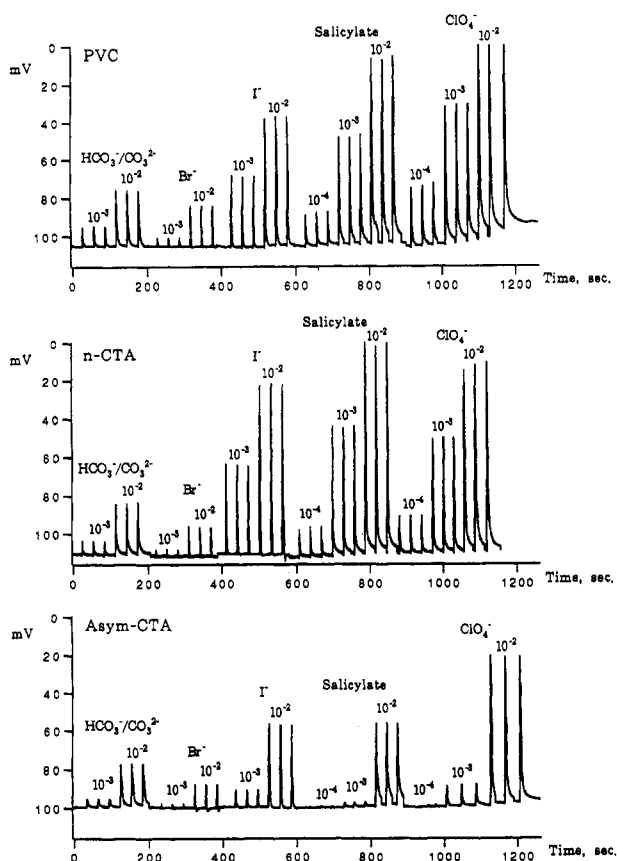


Figure 2. Potentiometric flow-injection anion response obtained with TFABB-doped carbonate-selective PVC, unmodified normal CTA, and asymmetric CTA membrane electrodes as the flow-through detector.

of the large concentration gradient developed between the outer sample solution and the aqueous layer (within the porous hydrophilic thin membrane) adjacent to the ion-sensing surface and because of the logarithmic responsive nature of the ion-selective membranes (i.e., only a small fraction of the hydrophobic anions can be excluded from the hydrophilic layer at higher anion concentrations).

The carbonate selectivity of the asymmetric membrane can be further enhanced by taking advantage of its slow response kinetics toward large anions. When the ion-selective electrode is employed directly in a wall-jet configuration, only a fraction of the response to the injected ions is observed because such flow-injection systems operate in a nonequilibrium mode. Thus, the resulting response signals (peaks) depend on the dynamic response characteristics of the ISE detector employed. Figure 2 clearly demonstrates this point. In this figure, the potentiometric flow-injection anion response peaks obtained with the PVC, CTA, and asymmetric CTA membrane electrodes are compared. The asymmetric membrane exhibited a significantly reduced response for large, lipophilic anions (e.g., iodide, salicylate, and perchlorate), compared with those of the conventional PVC- and unmodified CTA-matrix membranes, particularly at concentrations lower than 10^{-2} mol/L. For instance, in the static mode experiment, the asymmetric CTA and unmodified CTA membranes showed almost the same equilibrium selectivity for carbonate vs iodide (see Table I). However, the carbonate selectivity of the asymmetric membrane over iodide ion was significantly improved in this flow-injection detection system. Therefore, the selectivity of an asymmetric membrane can be most effectively enhanced by employing a nonequilibrium flow-through type of arrangement.

The asymmetric membrane gave a relatively large response for higher salicylate concentrations (e.g., 10 mmol/L; see Table

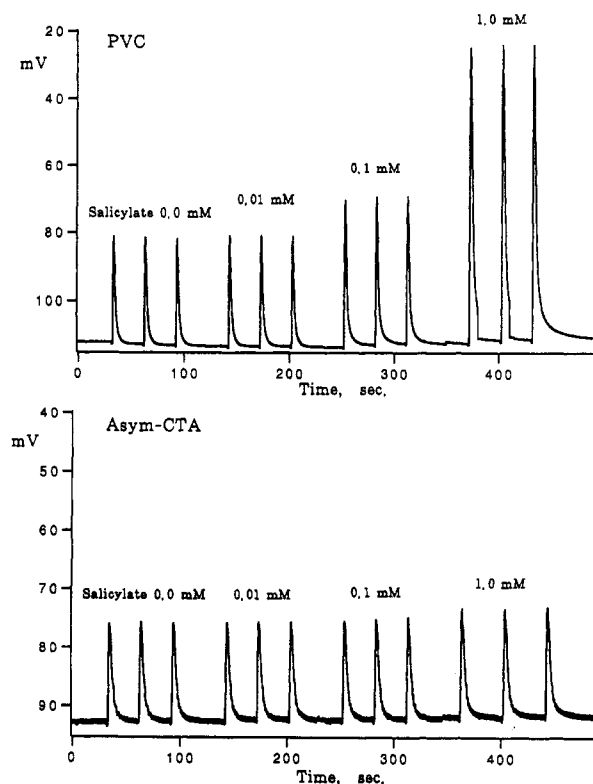


Figure 3. Flow-injection response of the PVC and asymmetric CTA membrane electrodes to injected 25 mmol/L carbon dioxide solutions containing varying salicylate concentrations (0.0, 0.01, 0.1, and 1.0 mmol/L).

II or Figure 2). Nonetheless, given that the normal level of total carbon dioxide for human serum is 21–31 mmol/L¹⁰ and the therapeutic levels of salicylate in serum range from 0.15 to 2.1 mmol/L,⁸ the asymmetric membranes may be used to overcome the salicylate interference problems in serum carbon dioxide measurements. Thus, a preliminary experiment was carried out by injecting several carbonate standard solutions (25 mmol/L) containing varying amounts of salicylate interferences (0.0, 0.01, 0.1, or 1.0 mmol/L; see Figure 3). When the asymmetric carbonate ion-selective electrode was used as the flow-through detector, the presence of salicylate interferences at a level comparable to the therapeutic level in the serum matrix did not significantly alter the observed potentiometric response to 25 mmol/L carbon dioxide. The PVC-based membrane exhibited a severe interference even from a concentration as low as 0.1 mmol/L salicylate.

Finally, the asymmetric carbonate-selective membranes were tested for their ability to quantitate total carbon dioxide in the human blood serum matrix. The membranes used in this study were prepared by using DOA as the plasticizer. This time, a static arrangement was employed to maximize the carbonate response signal and to investigate the feasibility of adapting the asymmetric membrane to the more desirable stop-flow arrangement. Using the setup described in the Experimental Section, the serum samples were added in the order shown in Figure 4. Curve A was to determine the amount of CO_2 species initially present in the reconstituted control serum sample. The PVC and asymmetric membranes resulted in different amounts of CO_2 , the PVC membrane yielding the higher level. Since the PVC-matrix membrane is known to suffer from various organic acid interferences present in the serum matrix, the value obtained with the asymmetric membrane was used. The initial amount of total CO_2 species present in the reconstituted serum was determined to be approximately 2.5 mmol/L. Thus, a quantity of carbon dioxide (in the form of NaHCO_3) was added to a serum

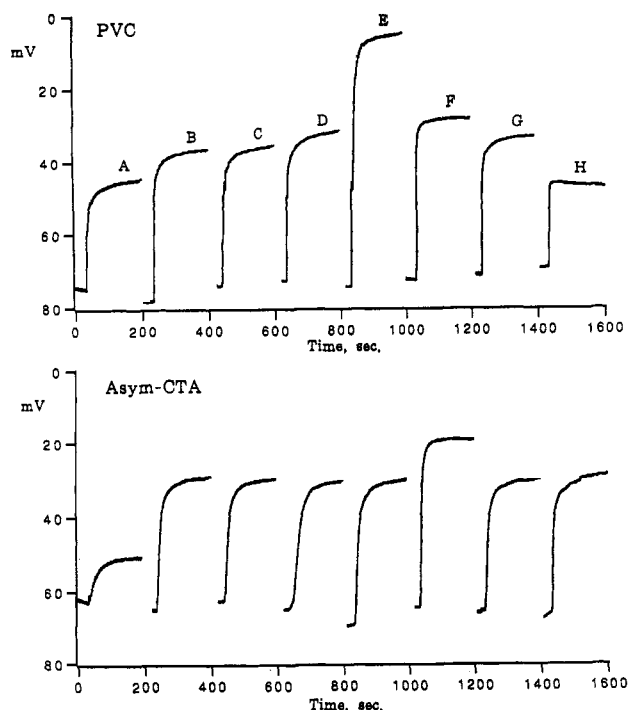


Figure 4. Potentiometric response of the PVC and asymmetric carbonate ion-selective membrane electrodes to added serum samples containing varying salicylate concentrations: (A) reconstituted control serum; (B) control serum containing 25 mmol/L CO_2 ; (C) control serum containing 25 mmol/L CO_2 and 0.01 mmol/L salicylate; (D) control serum containing 25 mmol/L CO_2 and 0.1 mmol/L salicylate; (E) control serum containing 25 mmol/L CO_2 and 1.0 mmol/L salicylate; (F) control serum containing 50 mmol/L CO_2 ; (G) control serum containing 25 mmol/L CO_2 ; (H) 25 mmol/L CO_2 in deionized water.

pool prepared with several reconstituted serum vials to make a final CO_2 concentration of 25 mmol/L. To portions of this serum sample varying amounts of salicylate were added. Indeed, the PVC-matrix membrane exhibited a huge interfering response to the serum sample containing 1.0 mmol/L salicylate (see curve E). On the other hand, the asymmetric

membrane showed virtually the same response to all 25 mmol/L CO_2 -containing serum samples with and without salicylate interference (compare curves B-E). Even an interference level of 1.0 mmol/L salicylate (curve E) did not alter the response of the asymmetric membrane. It was noted that the carbonate response slope (curve F vs curve G) of the PVC-matrix membrane was reduced possibly due to interfering ions initially present in the reconstituted serum sample. This is confirmed by comparing curve G with curve H. On the other hand, the asymmetric membrane maintained the expected slope in the serum matrix (curve F vs curve G), and the serum components did not interfere with the carbon dioxide measurement (curve G vs curve H).

In summary, a new concept of using asymmetric membrane technology to enhance the performance of the resulting membranes has been proposed. The asymmetric membranes are characterized by having a hydrophilic, porous layer on top of the sensing surface of the ion-selective layer and decreased interference from large, hydrophobic anions by the kinetic and exclusion mechanisms. The TFABB-doped asymmetric CTA carbonate ion-selective membrane electrodes exhibit a remarkably reduced response to hydrophobic anions such as salicylate or perchlorate, compared to the conventional PVC-matrix membrane electrodes. Given the results showing that the asymmetric membranes eliminate the salicylate interference when used in serum carbon dioxide measurements, it is likely that this approach will offer a relatively simple method to determine total CO_2 contents in various physiological samples.

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