Neutral Carrier Based Ion-Selective Electrode for the Determination of Total Calcium in Blood Serum

Peter Anker, Erich Wieland, Daniel Ammann, René E. Dohner, Robert Asper,[1] and Wilhelm Simon*

Department of Organic Chemistry, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland

A simple potentiometric determination of the total calcium concentration in human blood serum using a liquid membrane electrode is described. The bound calcium is displaced from its complexes through acidification of the serum sample with an acetate buffer to pH 3.5. A novel neutral carrier based Ca^{2+}-selective electrode showing a much higher rejection of H^+ ions in comparison to previously described carrier membranes as well as classical ion-exchanger membranes permits the Ca^{2+} determination at such a low pH value. A correlation of the total calcium concentrations determined by ion-selective electrodes with those obtained by atomic absorption spectrometry gives a residual standard deviation of ± 0.04 mM over the 1.48–3.00 mM Ca^{2+} range.

Through the introduction of Ca^{2+}-selective electrodes especially by Ross (*1*, *2*) the determination of ionized Ca^{2+} in blood serum became possible. Today improved electrodes based on organic phosphates (*3*, *4*) and on neutral carriers (*5*, *6*) are used in several routine instruments (*7–10*). Although the activity of the ionized Ca^{2+} is claimed to be physiologically more relevant (*11–13*), the determination of the total calcium concentration remains a useful diagnostic parameter (*14–16*). For the assessment of total calcium, e.g., the sum of the concentrations of the ionized Ca^{2+} and the calcium bound to proteins and various other complexing agents, atomic absorption spectrometry (AAS) and colorimetry predominate in daily use (see ref 13 and 14). AAS is rather complex for clinical environments and colorimetry necessitates an extended and involved sample treatment (*17–21*). Several components interfere with the colorimetric detection (*13*, *14*, *20*, *21*). Potentiometric sensors would offer an attractive alternative if it became possible to displace the bound calcium by a simple procedure. It has been shown that calcium can be displaced by hydrogen ions (*13*, *14*, *22–25*). This requires ion-selective electrodes with sufficient preference of Ca^{2+} over H_3O^+. Neutral carrier based Ca^{2+}-selective electrodes are unique in this respect (*5*). Here we report on the use of a newly developed membrane for the measurement of total Ca^{2+} in diluted, acidified human blood serum.

EXPERIMENTAL SECTION

Macroelectrode Systems. Cells of the type Hg,Hg_2Cl_2;KCl (satd.)|3 M KCl|sample solution||membrane||0.001 M $CaCl_2$;AgCl,Ag were used for macroelectrodes. The external macroreference electrode was a double junction calomel electrode Philips R 44/2 SD-1. Two different membranes were examined, one consisted of 1.0 wt % ligand ETH 1001, 0.7 wt % potassium tetrakis(*p*-chlorophenyl)borate (KTpClPB), 65.3 wt % *o*-nitrophenyl *n*-octyl ether (o-NPOE), and 33.0 wt % poly(vinyl chloride) (PVC) and the other of 3.4 wt % ligand ETH 1001, 2.0 wt % potassium tetrakis(*p*-chlorophenyl)borate, 62.9 wt % bis(2-ethylhexyl) sebacate (DOS), and 31.7 wt % poly(vinyl chloride). Both membranes were prepared by dissolving the four membrane components (total weight 180 mg) in about 2 mL of tetrahydrofuran and pouring the solution into a glass ring (23 mm i.d.) which rested on a glass plate. After overnight solvent evaporation, the resulting membrane, about 0.2 mm thick, was peeled away from the glass, and disks of 7 mm diameter were stamped out. The membranes were mounted in Philips electrode bodies type IS 561 (N.V. Philips, Gloeilampenfabrieken, Eindhoven, The Netherlands).

Flow-Through Electrode System. All measurements were carried out with cells of the type Ag,AgCl;1 M KNO_3 + 0.01 M KCl|sample solution||membrane||0.001 M $CaCl_2$;AgCl,Ag. The potential difference between the reference electrode and the calcium-selective electrode was measured differentially relative to the common electrode (Pt wire).

The flow-through electrode system used (see Figure 1) represents a further modification of two similar arrangements that were described earlier (*26*, *27*). In the figure only one ion-selective electrode is depicted though up to four such units were connected in series to perform the experiments described here. The electrode modules were machined from poly(methyl methacrylate) blocks. Reference electrode and common electrode were interconnected with a poly(tetrafluoroethylene) cone, while the other junctions were realized by means of silicone rubber O-rings. The whole arrangement was mounted inside an aluminum block. A peristaltic pump (Perpex Modell A, H. J. Guldener, Zurich, Switzerland) downstream of the common electrode aspirated the sample solution at a flow rate of 18 mL/h. A second peristaltic pump located upstream of the common electrode forced the reference electrolyte (0.9 mL/h) through a restriction into the sample solution. A cellulose ester filter (MF-Millipore, Millipore S.A., 67120 Molsheim, France) was used as restriction (see Figure 1). In order to eliminate electrostatic charging effects, the plastic pump covers were contacted with metal screens, all poly(tetrafluoroethylene) tubing was sleeved with wire mesh, and the screens, the mesh, and the aluminum block were grounded.

The membrane was composed of 3.4 wt % ligand ETH 1001, 2.1 wt % potassium tetrakis(*p*-chlorophenyl)borate, 63.5 wt % bis(2-ethylhexyl) sebacate, and 31.0 wt % poly(vinyl chloride). The preparation of such flow-through ion-selective electrodes is described in ref 26.

EMF Measurements. The measurements were performed at 21 ± 1 °C using for the macroelectrodes a unity gain buffer (Teledyne Philbrick, Model 1702) in combination with a Solartron A 240 digital voltmeter (see ref 28). The reference and the ion-selective electrodes of the flow-through system were provided with FET operational amplifiers AD 515 KH (Analog Devices, Norwood, MA) and the potential differences were measured differentially relative to the Pt-wire common. The analogue signal of the difference amplifier (see ref 26) was processed by a digital voltmeter (Solartron LM 1604 DC, Solartron Electronic Group Ltd., Farnborough, Hampshire, England; operating sensitivity ± 0.01 mV). EMF, time, and channel information were punched on paper tape (Facit 4070, Facit AB, Central Service Department, S-59700 Åtvidaberg, Sweden) for off-line processing on a Hewlett-Packard HP 9830 calculator system.

Selectivity Factors, Electrode Function. The selectivity factors were obtained by the separate solution method (SSM, 0.1 M chlorides (*29*)) for the macroelectrodes of both membrane compositions. The K^{Pot}_{CaH} values were also determined by the fixed primary ion method (FPM, 0.001 M calcium chloride (*30*) (see Figure 2)). The electrode function of the flow-through electrode system was determined by linear regression over the Ca^{2+} activity range 5×10^{-6} to 10^{-1} M (Figure 4). The experimental EMF values were corrected for changes in the liquid junction potential using

[1] Permanent address: Medico-chemical Central Laboratory, University Hospital, CH-8091 Zurich, Switzerland.

Reprinted from Analytical Chemistry, 1981, *53*, 1970.

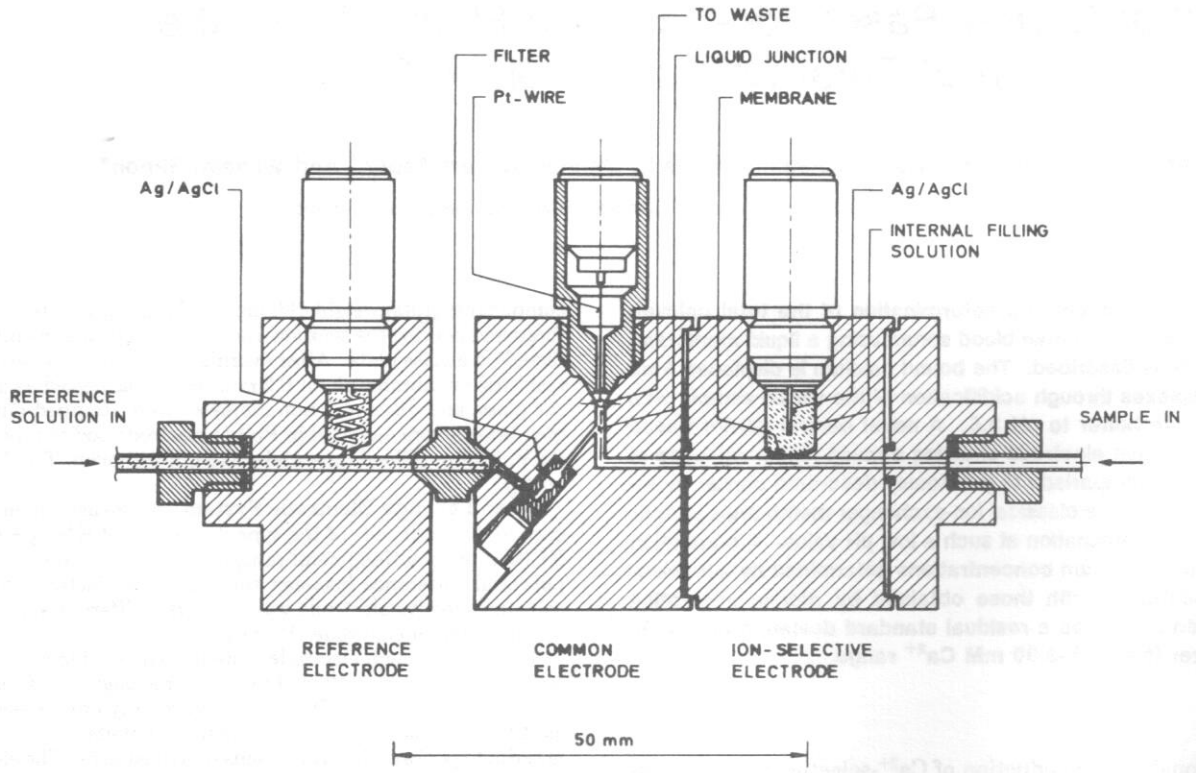

Figure 1. Schematic cross section of the flow-through electrode system.

the Henderson formalism (*6*, *31*). The activity coefficients were calculated according to the Debye–Hückel formalism and the parameters given in ref 6 and 31.

Blood Serum Measurements. The same measuring procedure was applied to both the macroelectrodes and the flow-through electrode system. The sera as well as the calibration solutions were diluted with a buffer solution to obtain a constant pH value of the sample solution of 3.5. The buffer contained 0.382 mol·L^{-1} acetic acid (Essigsäure 100%, p.a., E. Merck, Darmstadt, GFR) and 0.020 mol·L^{-1} sodium hydroxide (1 N Natronlauge, Titrisol, E. Merck, Darmstadt, GFR) yielding a pH value of 3.50. Calcium calibration solutions, solutions of 1.0, 2.0, and 9.0 mM Ca^{2+}, in the presence of 140 mM Na^+, 4 mM K^+, and 0.6 mM Mg^{2+} were diluted with a 20-fold volume of acetate buffer of the composition given above. The electrodes, including the macro reference electrode, were conditioned overnight in the diluted 2 mM Ca^{2+} calibration solution and before beginning the serum measurements the slopes were determined twice by measuring the three calcium calibration solutions. The 2 mM solution was then measured in alternation with a blood serum, also diluted with a 20-fold volume of acetate buffer. At the end of the serum measurements, the three Ca^{2+} calibration solutions were measured once more to check the electrodes. To reduce contamination of the sample solutions, we injected 10 air segments during sample changes.

The EMF readings were taken over periods of 20 min (macroelectrodes), respectively, of 10 min (flow-through system) after sample change. The response times, however, were considerably shorter (typical response time (*29*) observed in the flow-through electrode system: $t_{90} \leq 60$ s).

The evaluation of total calcium in the blood sera was performed with the following relation:

$$c_{Ca^{2+}} = 2 \times 10^{-\Delta E/s}$$

where $c_{Ca^{2+}}$ is the total calcium concentration [mmol·L^{-1}] in the blood serum, ΔE is the EMF difference [mV] of the cell assembly between the 2 mM Ca^{2+} calibration solution and the following serum sample, and s is the actual slope of the electrode.

Reagents. All aqueous electrolyte solutions were made from doubly quartz distilled water and salts of the highest purity available. Poly(vinyl chloride) (PVC S 704 hochmolekular) was obtained from Lonza AG, CH-3930 Visp, Switzerland, and bis-(2-ethylhexyl) sebacate (DOS, pract.) from Fluka AG, CH-9470

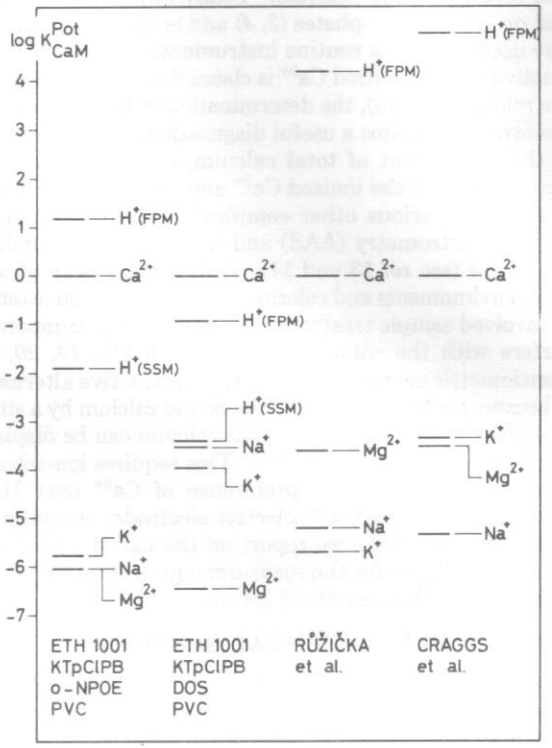

Figure 2. Selectivity factors, log K^{Pot}_{CaM} for membranes based on a neutral carrier (columns 1 and 2) or on organic phosphates (columns 3 (*3*) and 4 (*4*)) as determined by the separate solution method (*29*) or by the fixed primary ion method (FPM (*30*)) (for details see Experimental Section).

Buchs, Switzerland. The synthesis of (−)-(*R*,*R*)-*N*,*N*′-bis[(11-ethoxycarbonyl)undecyl]-*N*,*N*′,4,5-tetramethyl-3,6-dioxaoctanediamide (ligand ETH 1001) is described in detail in ref 32 and corresponds to the Ca^{2+} ligand Fluka No. 21192 (Fluka AG, CH-9470 Buchs, Switzerland), while *o*-nitrophenyl *n*-octyl ether (*o*-NPOE) and potassium tetrakis(*p*-chlorophenyl)borate

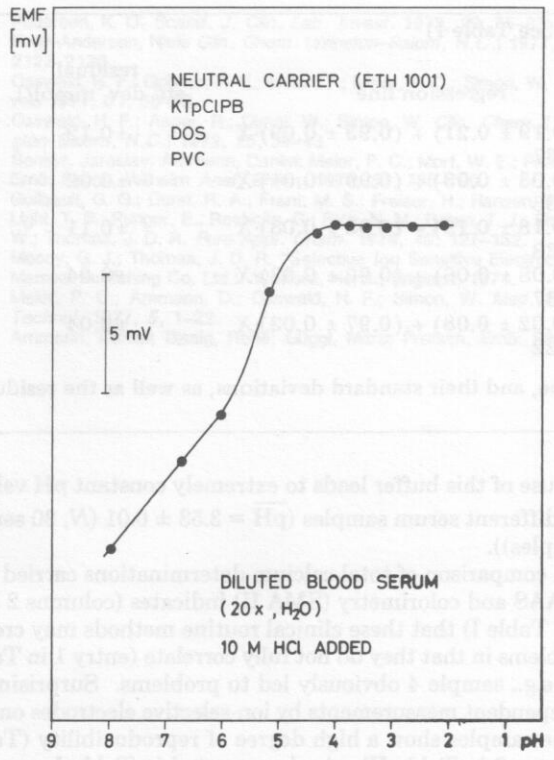

Figure 3. Displacement of calcium from its complexes by hydrogen ions in diluted blood serum investigated with a Ca^{2+}-selective macroelectrode.

(KTpClPB) were prepared according to ref 33 and 34, respectively. Tetrahydrofurane (THF, puriss. p.a.) was obtained from Fluka AG and was distilled prior to use. The sera were the remains of blood samples drawn at the University Hospital, Zurich, for routine investigations.

Atomic Absorption Spectrometry. For the determination of calcium by atomic absorption spectrometry, 100 μL of serum was diluted with 4.9 mL of a solution containing 0.1 M HCl and 0.76 mM CsCl as ionization suppressor. The measurements were carried out on a single-beam atomic absorption spectrometer (Elektrolytautomat FL6, Carl Zeiss, 7082 Oberkochen, GFR) employing an oxidizing air/acetylene flame as atom reservoir and a pulsed calcium/magnesium hollow cathode lamp. Behind the flame the light passed through an interference filter. The resulting signal (Ca) was processed by a photomultiplier and compared with the signal (Cs) of the dilution solution which was chosen as internal standard. The calibration of the instrument was performed with the CsCl solution and a reference solution containing 145 mM Na^+, 5 mM K^+, 2.5 mM Ca^{2+}, 1 mM Mg^{2+}, and 1.5 mM Li^+.

Colorimetry (SMA II (*19*)). The colorimetric determination of calcium was performed with a computer-controlled multichannel biomedical analyser SMA II (Technicon Instruments Corp., Tarrytown, NY). The serum sample was mixed with the 1.9-fold volume of a 0.3 M hydrochloric acid containing 0.25 wt % 8-hydroxyquinoline. This diluted serum was dialyzed into a 0.2 M HCl solution containing 0.25 wt % 8-hydroxyquinoline and 0.007 wt % cresolphthalein complexone. To eliminate heavy metal interference and to increase the pH, we added a solution of potassium cyanide in diethylamine to the dialyzate. The solution was then measured at 570 nm with a flow-through photometer; 1.1 mL of serum was needed, but 11 other parameters were simultaneously determined with this sample volume.

RESULTS AND DISCUSSION

Selectivity factors heavily depend on the method of determination as well as on the conditions chosen for a given method (*5*). This leads to discrepancies in the reported K^{Pot}_{CaM} values and makes difficult the comparison of selectivities of different Ca^{2+}-selective electrodes. Even by considering this fact the values K^{Pot}_{CaH} for the neutral carrier based membranes are significantly lower than for those based on organic phosphates. Values of K^{Pot}_{CaH} for some membranes (neutral carrier: columns 1 and 2; organic phosphates: columns 3 and 4) determined by different methods are given in Figure 2. This figure indicates that a PVC membrane with the carrier ETH 1001 and potassium tetrakis(*p*-chlorophenyl)borate in bis(2-ethylhexyl) sebacate is particularly suited for work in low pH sample solutions. By use of this electrode, the replacement of calcium in its complexes by hydrogen ions in diluted blood serum has been studied (Figure 3). To this end a serum sample was diluted with a 20-fold volume of water and concentrated hydrochloric acid was then added stepwise. The measured EMFs were corrected for the liquid junction potential (Henderson equation, ref 6 and 31) and for the decrease of the Ca^{2+} activity due to the addition of the acid (the activity coefficient γ_{Ca} is a function of the ionic strength; cf. Debye-Hückel formalism, ref 6 and 31). The addition of hydrochloric acid leads to increasing Ca^{2+} activities in the sample solution from pH 8 to 4. In the range of pH 4–2 the sample Ca^{2+} activity is unaffected. The bound calcium therefore seems to be fully replaced at a pH of about 3.5. The constant Ca^{2+} activity observed in the pH range 4–2 corroborates the high rejection of H^+ relative to Ca^{2+} by the electrode. This is very much in contrast to membranes based on organic phosphates. The best recently described membranes based on bis[4-(1,1,3,3-tetramethylbutyl)]phenyl phosphate are only claimed

Table I. Total Calcium Concentration in Blood Sera as Obtained by Different Methods (in mmol/L)

sample no.	colorimetry (SMA II)	AAS	potentiometry: macroelectrode	potentiometry: flow-through electrode system	
1	2.47	2.60	2.64	2.62	2.60
2[a]	2.60	2.64		2.63	2.62
3[a]	2.57			2.64	2.62
4	2.35	3.00	2.90	2.92	2.92
5	2.32	2.32	2.30	2.32	2.31
6	2.37	2.44	2.41	2.39	2.40
7	2.07	2.08	2.07	2.10	2.10
8	2.10	2.12	2.12	2.16	2.16
9	2.30	2.24	2.23	2.29	2.28
10	2.52	2.48	2.41	2.45	2.47
11	2.15	2.16	2.08	2.13	2.13
12	2.37	2.36	2.23	2.25	2.25
13	2.25	2.24	2.20	2.20	2.24
14	2.20	2.28	2.24	2.29	2.29
15	2.05	2.08	2.05	2.09	2.09
16	2.25	2.20	2.17	2.18	2.19
17	2.22	2.28	2.20	2.24	2.26
18	1.85	1.96	1.83	1.92	1.91
19	2.27	2.28	2.23	2.26	2.27
20	2.42	2.40	2.31	2.32	2.34
21	2.12	2.16	2.10	2.11	2.10
22	2.20	2.32	2.26	2.26	2.27
23	1.38	1.48	1.48	1.48	1.49
24	2.32	2.28	2.22	2.28	2.27
25[a]	2.27	2.20		2.25	2.25
26[a]	2.42	2.40		2.36	2.34
27[a]	2.30	2.24		2.28	2.26
28[a]	2.34	2.32		2.31	2.31
29[a]	2.00	2.00		1.97	1.99
30[a]	2.47	2.44		2.44	2.46
31[a]	2.49	2.48		2.48	2.44
32[a]	2.52	2.44		2.43	2.41
33[a]	2.32	2.32		2.32	2.37
34[a]	2.39	2.36		2.36	2.36
35[a]	2.44	2.44		2.39	2.39
36[a]	2.20	2.16		2.13	2.11
37[a]	2.37	2.36		2.35	2.33
38[a]	2.12	2.16		2.16	2.12

[a] Not sufficient serum was available to carry out all determinations in columns 3 and 4.

Table II. Correlations[a] among Different Assays for Total Calcium (See Table I)

methods	regression line	residual std dev, mmol/L
AAS (Y) vs. colorimetry (SMA II: X)	$Y = (0.19 \pm 0.21) + (0.93 \pm 0.09) \cdot X$ $n = 37$	±0.12
potentiometry (two electrodes, X and Y, of the flow-through system)	$Y = (0.03 \pm 0.03) + (0.99 \pm 0.01) \cdot X$ $n = 38$	±0.02
potentiometry (flow-through system: Y) vs. colorimetry (SMA II: X)	$Y = (0.18 \pm 0.19) + (0.93 \pm 0.08) \cdot X$ $n = 38$	±0.11
potentiometry (flow-through system: Y) vs. AAS (X)	$Y = (0.08 \pm 0.06) + (0.96 \pm 0.03) \cdot X$ $n = 37$	±0.04
potentiometry (macroelectrode: Y) vs. AAS (X)	$Y = (0.02 \pm 0.08) + (0.97 \pm 0.03) \cdot X$ $n = 22$	±0.04

[a] The regression lines are given by the intercept (mmol/L), the slope, and their standard deviations, as well as the residual standard deviation (mmol/L).

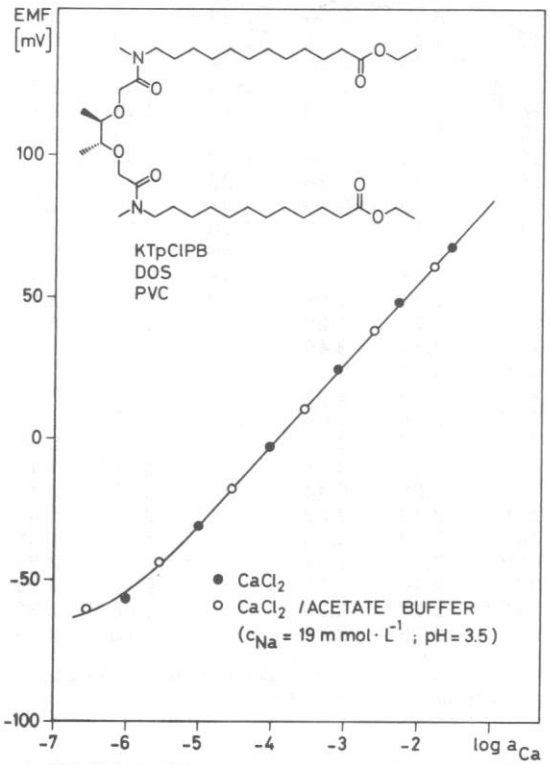

Figure 4. Electrode functions measured with the flow-through electrode system.

to have no hydrogen ion interference in 10^{-3} M Ca^{2+} solutions at pH values above 4.9 (*4*). They are therefore unsuitable for the application discussed here.

Obviously the components used to acidify the blood serum should not interfere with the Ca^{2+}-selective electrode and should not complex Ca^{2+}. A sodium acetate buffer was most promising since it does not change the electrode response as compared to pure $CaCl_2$ solutions (see Figure 4). The slope of the function obtained with pure $CaCl_2$ solutions is 28.8 ± 0.1 mV (standard deviation, $N = 5$) in the Ca^{2+} range 5×10^{-6} to 10^{-1} M. The same solutions diluted with a 20-fold volume of acetate buffer (see Experimental Section) yield a response with a slope of 28.8 ± 0.1 mV in the same activity range (theoretical value, 29.2 mV (21 °C)). In both cases the detection limit (*29*) is about 7×10^{-7} M Ca^{2+}. By use of the buffer described in the Experimental Section, a dilution by a factor of about 20 of the blood serum leads to a rather constant ionic strength of the final sample solution given mainly by the Na^+ present. The resulting calcium concentrations (0.7×10^{-4} to 1.9×10^{-4} M Ca^{2+}) are well above the detection limit of the electrode system (about 7×10^{-7} M Ca^{2+}) at the prevailing Na^+ background (see Figure 4). In addition, the use of this buffer leads to extremely constant pH values for different serum samples ($\overline{pH} = 3.53 \pm 0.01$ (N, 30 serum samples)).

A comparison of total calcium determinations carried out by AAS and colorimetry (SMA II) indicates (columns 2 and 3 in Table I) that these clinical routine methods may create problems in that they do not fully correlate (entry 1 in Table II), e.g., sample 4 obviously led to problems. Surprisingly, independent measurements by ion-selective electrodes on the same samples show a high degree of reproducibility (Table I, entry 2 in Table II). As documented in Table I, e.g., for sample 4 (see also Table II), the colorimetric procedure is responsible for the relatively large discrepancies. Although flow-through electrode systems are especially suited for automation (*27*, *35*, *36*), conventional dip type ion-selective macroelectrodes may be used as well to obtain highly reliable results (column 4 in Table I and entry 5 in Table II). Entries 4 and 5 in Table II further indicate that the employed calibration procedure leads to highly accurate results.

LITERATURE CITED

(1) Ross, James W., Jr. *Science* **1967**, *156*, 1378–1379.
(2) Ross, James W., Jr. In "Ion-Selective Electrodes"; Durst, R. A., Ed.; National Bureau of Standards: Washington, DC, 1969; Chapter 2; NBS Spec. Publ. (U.S.) No. 314.
(3) Růžička, J.; Hansen, E. H.; Tjell, J. Chr. *Anal. Chim. Acta* **1973**, *67*, 155–178.
(4) Craggs, A.; Moody, G. J.; Thomas, J. D. R. *Analyst (London)* **1979**, *104*, 412–418.
(5) Simon, W.; Ammann, D.; Oehme, M.; Morf, W. E. *Ann. N.Y. Acad. Sci.* **1978**, *307*, 52–70.
(6) Meier, P. C.; Ammann, D.; Morf, W. E.; Simon, W. In "Medical and Biological Applications of Electrochemical Devices"; Koryta, J., Ed.; Wiley: Chichester, New York, Brisbane, Toronto, 1980; Chapter 2.
(7) Instruction Manual, Nova Biomedical, Model NOVA 2, Newton, MA.
(8) Instruction Manual, Applied Medical Technology, Inc., AMT Clin ± Ion, Menlo Park, CA.
(9) Instruction Manual, AVL GmbH, Model AVL 980, 8020 Graz, Austria.
(10) Instruction Manual, Orion Biomedical, Model SS-20, Cambridge, MA, 1975.
(11) McLean, Franklin C.; Hastings, A. Baird *J. Biol. Chem.* **1934**, *107*, 337–350.
(12) Copp, D. Harold *J. Endocrinol.* **1969**, *43*, 137–161.
(13) Robertson, William G.; Marshall, Robert W. *CRC Crit. Rev. Clin. Lab. Sci.* **1979**, *11*, 271–304.
(14) Lott, John A. *CRC Crit. Rev. Anal. Chem.* **1972**, *3*, 41–64.
(15) Ladenson, Jack H.; Bowers, George N., Jr. *Clin. Chem. (Winston-Salem, N.C.)* **1973**, *19*, 575–582.
(16) Paschen, K. "Die Bestimmung des Calciums und seiner Fraktionen im Serum. Methoden, Normalwerte und klinische Bedeutung"; Georg Thieme Verlag: Stuttgart, 1975.
(17) Kessler, Gerald; Wolfman, Morris *Clin. Chem. (Winston-Salem, N.C.)* **1964**, *10*, 686–703.
(18) Gitelman, Hillel J. *Anal. Biochem.* **1967**, *18*, 521–531.
(19) Instruction Manual, Technicon Instruments Corp., SMA II, Tarrytown, NY.
(20) Gindler, E. Melvin; King, John D. *Am. J. Clin. Pathol.* **1972**, *58*, 376–382.
(21) Küffer, H.; Degiampietro, P. *Clin. Chem. (Winston-Salem, N.C.)* **1975**, *21*, 961.
(22) Loken, H. F.; Havel, R. J.; Gordan, G. S.; Whittington, S. L. *J. Biol. Chem.* **1960**, *235*, 3654–3658.
(23) Peterson, N. A.; Feigen, G. A.; Crismon, J. M. *Am. J. Physiol.* **1961**, *201*, 386–392.

(24) Pedersen, K. O. *Scand. J. Clin. Lab. Invest.* **1972**, *29*, 75–83.
(25) Fogh-Andersen, Niels *Clin. Chem.* (*Winston-Salem, N.C.*) **1977**, *23*, 2122–2126.
(26) Osswald, H. F.; Dohner, R. E.; Meier, T.; Meier, P. C.; Simon, W. *Chimia* **1977**, *31*, 50–52.
(27) Osswald, H. F.; Asper, R.; Dimai, W.; Simon, W. *Clin. Chem.* (*Winston-Salem, N.C.*) **1979**, *25*, 39–43.
(28) Senkyr, Jaroslav; Ammann, Daniel; Meier, P. C.; Morf, W. E.; Pretsch, Ernö; Simon, Wilhelm *Anal. Chem.* **1979**, *51*, 786–790.
(29) Guilbault, G. G.; Durst, R. A.; Frant, M. S.; Freiser, H.; Hansen, E. H.; Light, T. S.; Pungor, E.; Rechnitz, G.; Rice, N. M.; Rohm, T. J.; Simon, W.; Thomas, J. D. R. *Pure Appl. Chem.* **1976**, *48*, 127–132.
(30) Moody, G. J.; Thomas, J. D. R. "Selective Ion Sensitive Electrodes"; Merrow Publishing Co. Ltd.: Watford, Herts., England, 1971.
(31) Meier, P. C.; Ammann, D.; Osswald, H. F.; Simon, W. *Med. Prog. Technol.* **1977**, *5*, 1–22.
(32) Ammann, Daniel; Bissig, René; Güggi, Marc; Pretsch, Ernö; Simon, Wilhelm; Borowitz, Irving J.; Weiss, Louis *Helv. Chim. Acta* **1975**, *58*, 1535–1548.
(33) Horning, E. C. In "Organic Synthesis"; Wiley: New York, 1955; Vol. III, p 140.
(34) Cassaretto, Frank P.; McLafferty, John J.; Moore, Carl E. *Anal. Chim. Acta* **1965**, *32*, 376–380.
(35) Schindler, J. G.; Dennhardt, R.; Simon, W. *Chimia* **1977**, *31*, 404–407.
(36) Simon, W.; Ammann, D.; Osswald, H. F.; Meier, P. C.; Dohner, R. E. In "Advances in Automated Analysis. Technicon International Congress 1976"; Mediad Inc.: Tarrytown, NY, 1977; Vol. 1.

RECEIVED for review May 26, 1981. Accepted July 13, 1981. This work was partly supported by the Swiss National Science Foundation. P.A. thanks Rhône Poulenc as well as Orion Research for a grant.