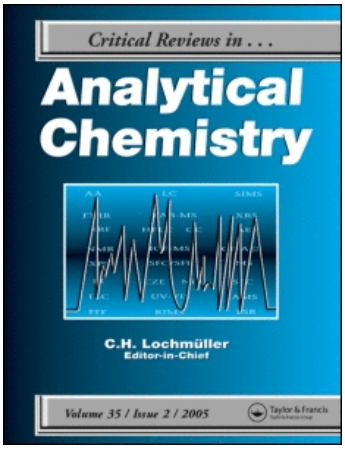

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Recent Advances in Pharmaceutical Analysis with Potentiometric Membrane Sensors

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Recent Advances in Pharmaceutical Analysis with Potentiometric Membrane Sensors

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ABSTRACT: The development and applications of ion-selective membrane electrodes continue to provide excitement in expanding areas of analytical chemistry, including analytical pharmaceutical research and analysis, because these sensors offer the advantages of simple design, construction, and manipulation, reasonable selectivity, fast response time, applicability to colored and turbid solutions, and possible interfacing with automated and computerized systems. The combination of membrane sensors with flow injection analysis (FIA) for the determination of various organic ions of biological interest (e.g., drugs) is a new promising area with many applications. This review covers the material that is of interest to those who deal with ion-selective electrodes in organic and pharmaceutical analysis.

KEY WORDS: ion-selective electrodes, membrane sensors, drugs, pharmaceutical analysis, dissolution, pharmacokinetics.

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Drug-Substances Assayed by Membrane Sensors

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INDEX (continued)

I. INTRODUCTION

New requirements and the development of adequate analytical methods for the determination of various drugs and pharmaceuticals in complex samples led many researchers to describe and apply new potentiometric techniques involving ion-selective electrodes. In many instances, both *British Pharmacopeia*[1] and *United States Pharmacopeias*[2] give assay methods requiring pretreatment steps such as repeated extractions from acidic or alkaline solutions. These methods, besides being tedious, suffer, in many cases, from severe interferences by various related compounds. Many of the sophisticated methods (nuclear magnetic resonance spectroscopy, circular dichroism, fluorescence polarization immunoassay, gas–liquid chromatography, gas chromatography–mass spectrometry, high pressure liquid chromatography, radioimmunoassay, etc.) require several time-consuming manipulation steps, expensive equipment, and special training.

Ion-selective electrodes have become important and reliable devices for chemical, pharmaceutical, and biomedical analysis; they are inexpensive, easy to use, require simple maintenance, and can offer a high speed for the assay procedure. Potentiometric analysis with ion-selective electrodes becomes even more attractive and faster when the elec-

trodes are incorporated in a mechanically stable manner within a flow injection analysis (FIA) system.

One of the very common existing principles for the construction of ion selective membranes, sensitive to various drugs compounds is the addition of a lipophilic ion-pair complex into a highly plasticized membrane. Since Baum et al.[3-6] succeeded in the construction of an acetylcholine-selective electrode in the seventies which contained potassium-tetrakis-(4-chlorophenyl)-borate as an ion exchanger and DOP as a solvent mediator (plasticizer), many other organic sensitive electrodes have been reported.[7-15]

The high selectivity characteristics of many neutral carrier ligands are of much interest to analytical chemists and have been exploited in the development of highly selective K^+, Ca^{2+}, Na^+, etc. membrane sensors.[16] Among the few studies reported to date on the use of neutral carrier ligands for organic ions are the potentiometric sensors for drugs such as guanidine, adenosine, amphetamine, primaquine[10,11] (see also, Sections III.A and III.MM). In these complexes, interaction between host and guest is mainly achieved by hydrogen bonding and dipole-induced forces.

Developments in pharmaceutical analysis with ion-selective electrodes have enabled the activity of various organic cations and anions to be measured directly and selectively and, in most instances, without prior separation of the active substance from the formulation matrix. It is usually possible to develop methods for the determination of drug substances in pharmaceutical preparations that would need only a pre-dilution step (e.g., injectable preparations) or dissolution of tablets in the measuring solvent. The development of a potentiometric ion-selective electrode that can be calibrated to read the enantiomeric purity of a chiral analyte is an attractive target. Prompted by the use of peralkylated cyclodextrins as gas-chromatographic or HPLC chiral stationary phases[17], the potential of peroctylated cyclodextrins in electrochemical sensors for a range of chiral molecules incorporating aryl rings has been recently investigated with promising results (see Section III.T).

Other immediate fields of application of membrane electrodes are in the determination of tablet content uniformity and in dissolution profile studies. In many instances, the content uniformity test is preferred to the assay of a composite sample, and measurements can be carried out more rapidly than those of the assay of a composite sample. If the accuracy of the assay is satisfactory, the mean value of the content uniformity test can be used as the assay result. The desirability of an *in vitro* test that can adequately reflect the physiological availability of solid dosage forms of drugs is now recognized. The measurement of a parameter that is related to the rate of dissolution of a solid has been suggested as a more realistic variable, and this has led to numerous papers describing different methods and equipment for monitoring dissolution tests.[11,18] The advantage of the electrode technique for performing such tests is that the selective electrode can monitor continuously the concentration of the active ingredient in the standardized dissolution cells.

The current review is a survey of the literature on the use of ion-selective electrodes in pharmaceutical research and analysis that has appeared between summer 1989 and December 1992. The material used in this review was obtained from the major analytical, electroanalytical, and pharmaceutical journals and hand search of *Chemical Abstracts* and *Analytical Abstracts* to yield the remaining important publications. The format and rules for selecting the references are similar to the ones used in the previous reviews[8,10] in this series published by the former *Selective Electrode Reviews* journal.

II. BRIEF THEORETICAL BACKGROUND

Ion-selective electrodes may be roughly classified in the following way, according to the physical state of the substances (the electroactive materials) that form the electrode membrane:

1. Ion-selective electrodes with solid membranes. The membrane may be homoge-

neous as in a monocrystal, a sparingly soluble, ionic crystalline substance, or a glass, which is considered to be a solid because of the immobility of the ionic components. Alternatively, the membrane may be heterogeneous, by the incorporation of the electroactive substance within an inert matrix.

2. Ion-selective electrodes with liquid membranes. Here the electrode membrane is represented by an organic liquid immiscible with water. The organic liquid contains a charged electroactive substance that serves as "sites" for exchange of ions between membrane and solution. The membrane is responsive and may be selective for the exchangeable ions.

From the practical point for organic and pharmaceutical research and analysis, electrodes with mobile charged sites are most important. These include:

1. *Positively charged*, hydrophobic cations (e.g., those of quaternary ammonium salts or salts of substitutionally inert transition metal complexes such as derivatives of 1,10-phenanthroline), which, when dissolved in a suitable organic solvent and held on an inert support (e.g., Millipore filter or PVC), provide membranes which are sensitive to changes in the activity of anions. The hydrophobic cations are "trapped", mobile sites that are mainly confined to the membrane phase.
2. *Negatively charged*, hydrophobic anions (e.g., of type $[RO]_2PO_2^-$, tetra-*p*-chlorophenyl borate, dinonylnaphthalene sulfonate), which, when dissolved in a suitable organic solvent and held in an inert support (e.g., Millipore filter or PVC), provide membranes that are sensitive to changes in the activities of cations.
3. *Uncharged "carrier"* electrodes based on solutions of molecular complexing agents of cations (e.g., ion-dipole formers: antibiotics, macrocyclic compounds, or other sequestering agents) and anions (e.g., adduct formers: organotin compounds, activated carbonyl compounds, and some porphyrins).
4. *Hydrophobic ion-pair* electrodes of plasticized polymers (e.g., PVC) containing a dissolved hydrophobic ion pair, and "ion association complex" (e.g., a cation drug tetraphenylborate, or tetraalkylammonium surfactant anion) respond to component ion activities in bathing electrolytes. Responses can be Nernstian to bathing electrolytes of the cation drug chloride, or sodium tetraphenylborate.

A. Ion Association Drug Sensors

Design principles for sensors of ionic drugs (item 4 above) follow from application of the concepts used to describe the theoretical voltage-activity response relations for the various primary ion-sensitive sensors. The so-called "trapped sites" of the mobile-site, liquid ion-exchanger electrodes belong to a category of compounds known as ion association extractants. Examples are long-chain diesters of phosphoric acid and tricaprylylmethylammonium (Aliquat 336S) ions. The latter cation was studied extensively by Freiser and co-workers[19-21] in the design of anion sensors. The former were the original class of hydrophobic, liquid ion-exchanger species used in the Ca^{2+} sensors of Ross.[22]

In 1970, Higuchi et al.[23] and Liteanu and Hopirtean[24] introduced liquid membrane electrodes responsive both to organic and inorganic ions, including both cations and anions of the species constituting the membrane. Because they responded to bathing activities of inorganic counterions (as expected) and also to any site species dissolved in the bathing electrolyte (not expected), these electrodes seemed to be neither conventional mobile-site, liquid ion-exchanger nor neutral carrier-based. At the 1973 IUPACInternational Symposium on Ion-Selective Electrodes, J. R. Cockrell, Jr. presented an unpublished example of liquid membrane responsive to *both organic cations and organic anions*, not unlike responses of silver halide membranes, i.e., the membrane

electrode responded to its component cations or anions depending on which was in excess in the bathing electrolyte.

When a membrane contained a hydrophobic salt M^+X^- in a solvent-plasticized membrane, then a response was found for bathing salts M^+Cl^- or Na^+Y^- in solution. A membrane containing a detergent ion pair was responsive to either cationic or anionic detergent species in solution, depending on which was in excess. At the time, this effect seemed anomalous, because the idea of "trapped" sites was violated. It is now known that hydrophobic liquid membrane component ions are not really "trapped", but only thoroughly partitioned into the membrane by a favorable salt extraction equilibrium. Nevertheless, there must always be some salt remaining in the bathing solution, and this residual concentration determines the detection limit of the electrode in the same way that the solubility product of a silver halide membrane determines its ultimate response sensitivity.

B. Conditions for Two Opposite-Charge Ion Responses

The local interfacial equilibrium principles based on equality of electrochemical potential of each ion that reversibly equilibrates across an immiscible interface, were available for analysis of drug sensing electrodes prior to 1970. However, the theory and consequences for membranes based on ion association or ion-pair hydrophobic salts partitioned into membranes, were worked out later than the theory for other primary electrodes.[25,26] To develop an interfacial potential difference, two ions, M^+ and Y^-, that partition are generally involved, although in many simplified cases, it appears that only ions of one sign are partitioning. This condition can be considered necessary, but not sufficient to produce a sensor with good response for a single ionic species. The reason is that salt partitioning, e.g., extraction of equal concentrations of M^+ and Y^-, produces a potential difference (pd) that is independent of bathing salt concentration in either phase.

To develop a pd dependent solely on a single ion activity, say M^+, three ions are required: M^+, X^-, and Y^-, of which Y^- is very oil-soluble, X^- is mainly water soluble, and M^+ is soluble in both phases. The salt MY is typically an organic ion association species that may be isolated and dissolved in an organic solvent, or prepared *in situ* by extraction. The anion is typically picrate, tetraphenylborate, or an even more oil-soluble species. The salt MX, where $X^- = Cl^-$, is the sample whose M^+ activity is to be measured at variable values in the aqueous bathing electrolyte. When MX is varied, the interfacial pd is overall S-shaped (millivolts (mV) vs. log[MX]), but contains a linear section, the so-called Nernstian region.

It is merely an extension of these ideas to demonstrate the conditions that the same membrane, containing MY, should also be responsive, in a Nernstian fashion, to Y^- activities in solution. These conditions again call for consideration of three ions: M^+, Y^-, and N^+. The salt NY is the aqueous sample whose Y^- activity is to be measured. N^+ is typically a hydrophilic cation such as Na^+. When aqueous NY activity is varied, the interfacial pd is again S-shaped (mV vs. log[NY]). These responses are illustrated from a theoretical calculation in Figure 1. The assumed extraction parameters are given in the following section. The similarities with silver halide membrane electrode responses are summarized below:

Saturated salt AgBr (aq.)

Anion responsive
(aq.) Na^+Br^- at $Cs > K_{so}^{1/2}$
Cation responsive
(aq.) $Ag^+NO_3^-$ at $Cs > K_{so}^{1/2}$

Partitioned salt MY (very oil-soluble)

Anion responsive
(aq.) Na^+Y^- at $Cs > C_{MY}$(aq.)
Cation responsive
(aq.) M^+Cl^- at $Cs > C_{MY}$(aq.)

It is the hyperbolic relations $(Ag^+)(Br^-) = K_{so}$ and $(M^+)(Y^-)_s = (M^+)(Y^-)_m/K^2$ that

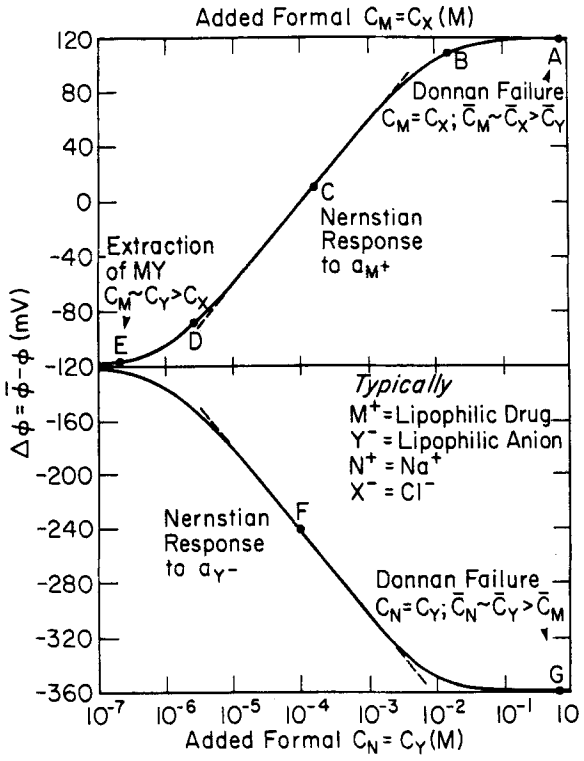


FIGURE 1. Calculated response curves for drug membrane sensors. Upper curve, response to M^+; lower curve, response to Y^- (single-ion partition coefficients were chosen to be $k_X = 10^{-2}$; $k_M = 10^2$; $k_Y = 10^6$, and $k_N = 10^{-2}$). (Reproduced from Buck, R.P.; Cosofret, V.V. Design of drug sensors: principles and practice, in *Fundamentals and Applications of Chemical Sensors;* D. Schuetzle and D. Hammerle, Eds.; American Chemical Society: Washington, D.C., 1986; p 365. With permission.)

provide the basic analogy between the two kinds of systems. In the latter, K^2 is the ionic salt partition coefficient relating membrane and bathing solution activities at an equilibrium interface. The latter form can also be derived for insoluble salt membranes. However, the salt phase ion activities are constant and so are hidden in the value of the solubility product K_{so}.

C. Local (Interface) Equilibrium Theory

Each ion M^+, X^-, and Y^- will generally have different energies in water and in the organic phase within the solvent-plasticized membrane. The plasticizer is usually a low dielectric constant compound such as dibutylphthalate or dioctyladipate. The partition free energy for each ion is written

$$\Delta G_i = \mu^0_{im} - \mu^0_{is} = -RT \ln k_i \qquad (1)$$

for the reaction

species i (soln. s)

$$= \text{species } i \text{ (org. membrane } m) \qquad (2)$$

is a measure of the intrinsic ionic oil-solubility of an ion, where k_i is the single-ion partition coefficient and m and s denote organic membrane and aqueous bathing phase quantities. For oil-soluble (hydrophobic) ions, ΔG is more negative and k_i is larger than for water-soluble (hydrophilic) ions.

D. Potential Theory

The interfacial potential difference (pd) for each interface is found from the ionic partition equilibrium. The value must be the same whether calculated from electrochemical potentials of M^+, N^+, X^-, or Y^-:

$$\Delta\phi = \phi_m - \phi_s = \frac{RT}{z_i F} \ln \frac{k_i a_{is}}{a_{im}} \qquad (3)$$

However, for convenience in calculation, the interfacial pd is determined from those species whose activities are known or easily calculated. From the upper part in Figure 1, in the linear range, MX is predominantly in water while MY is predominantly in the organic phase, since $k_X \ll k_Y$. Consequently, M^+ activities are known and used in Eq. (3) to give

$$\Delta\phi = \frac{RT}{z_i F} \ln \frac{k_M a_{Ms}}{a_{Mm}} = \Delta\phi^0 + \frac{RT}{F} \ln a_{Ms} \qquad (4)$$

This is a Nernstian response over a wide activity range. However, at very low MY bathing activities, the pd becomes insensitive to further decreasing M^+ activities and the response levels off at a value

$$\Delta\phi = \frac{RT}{2F} \ln \frac{k_M}{k_Y} \quad \text{for low MX} \qquad (5)$$

while at very high MX activities (generally only seen when $X^- = I^-$, NO_3^-, or ClO_4^-), the pd again levels off since Donnan exclusion is violated by encroaching X^- entering the organic phase and

$$\Delta\phi = \frac{RT}{2F}\ln\frac{k_M}{k_X} \quad \text{for high MX} \qquad (6)$$

By the same analysis, a single salt MY partitioned gives a constant potential in Eq. (5). These two limiting values are shown in Figure 1, points A and E. Likewise, Donnan failure upon addition of excess aq. NY gives a constant negative limit of

$$\Delta\phi = \frac{RT}{2F}\ln\frac{k_N}{k_Y} \qquad (7)$$

Details of the curvature regions in Figure 1 have been given in Reference 25.

E. Detection Limit and Selectivity

The lower detection limit for ions M^+ and Y^- is often given as the intersection of the Nernstian region with the limiting potential of Eq. (7). This value depends on the membrane loading MY_m and is given by

$$\frac{a_{Y_s}(\text{lower limit})}{aY_M} = \frac{a_{M_s}(\text{lower limit})}{aY_m} = (k_M k_Y)^{-1/2} \qquad (8)$$

which is 10^{-6} M in Figure 1. The total range of available potentials for M^+ measurements is determined by the two limiting potentials in Eqs. (5) and (6). This is the availability "window" for M^+:

$$\Delta\phi = \frac{RT}{2F}\ln\frac{k_Y}{k_X} \qquad (9)$$

There is also a "window" for Y^- measurements.

The definition of selectivity of the electrode for two ions of the same sign requires consideration of the responses to MY and IX when the membrane is initially loaded with MY. M^+ is the principal ion and I^+ is the interfering ion. Then the ion-exchange equilibrium requires formation of both MY and IY in the membrane. Consequently, the interfacial pd is related to both a_M^+ and to a_I^+ according to

$$\Delta\phi = \frac{RT}{F}\ln\left[\frac{k_M}{f_{Mm}(Y_m)}\left(a_M + \frac{k_I f_M}{k_M f_I}a_I\right)\right] \qquad (10)$$

The interfacial pd selectivity coefficient, the factor multiplying a_I is determined by the ion-exchange ratio $K_{iex} = k_I/k_M$, by the activity coefficient ratio f_M/f_I in solution, and by the mobility ratio, after the internal diffusion potential contribution is added. Interferences should correlate with the ion-exchange constant, which can be determined from salt extraction coefficients $k_I k_X/k_M k_X$ for a series of positive drug cations, comparing salts with common anions. This result is well documented in early ion-selective electrode response studies.[27,28] A commentary on this topic is given by Koryta[29] and Koryta and Stulik.[30] For drug electrodes there is a correlation of improved selectivity of N-based drugs with the extent of nitrogen substitution:

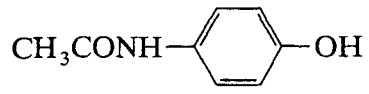
$$RNH_3^+ < R_2NH_2^+ < R_3NH^+ < R_4N^+$$

in which quaternary drugs of the same carbon number are most sensitively detected. Omitted from this elementary theory are effects of plasticizer and ion pairing, but these are mentioned in more detail in Reference 11.

III. DRUG-TYPE SUBSTANCES

A. Acetaminophen

$C_8H_9NO_2$ (MM = 151.2)



Therap. category: analgesic, antipyretic

Discussion and Comments

A flow injection (FI) kinetic potentiometric method for the determination of acetaminophen and other phenolic and hydrazino drugs (e.g., isoniazid and isoxsuprine; see Sections III.BB and III.CC, respectively) was described by Apostolakis et al.[31] The method is based on monitoring the liberation of fluoride with a fluoride-selective electrode as a detector during the reaction of the drug with 1-fluoro-2,4-dinitrobenzene (FDNB). It is well known that this reagent is selective for various organic functional groups (i.e., amines, amino acids, phenols, thiols, hydrazines, hydrazides, and azides) and previously served for the determination of other organic compounds, including several drugs, by kinetic potentiometric methods.[32-35]

The optimized manifold shown in Figure 2 was used for the automated kinetic potentiometric determination using FDNB. The combination fluoride electrode (Orion 96-09) was connected to the pH/pIon-meter unit of the electrometer-recorder system. In order to automate the method, the following aspects were taken into account[31,32]:

1. The reaction with the analyte is subject to base catalysis and it is favored by alkaline solutions.
2. FDNB reagent undergoes hydrolysis in the alkaline solutions with F^- production.
3. The optimum pH range for measurements with the fluoride electrode is 5 to 5.5.

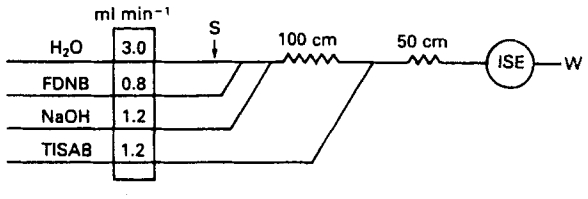


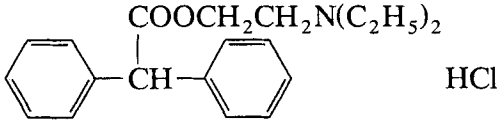
FIGURE 2. Flow injection manifold designed for kinetic-potentiometric determinations using a fluoride selective flow-through electrode: S is sample introduction; W is waste. (Reproduced from Apostolakis, J.C. et al. *Analyst* **1991**, *116*, 233. With permission from The Royal Society of Chemistry.)

As a consequence, the FI manifold (Figure 2) was constructed in such a way that the reaction takes place in the first coil (100 cm) in an alkaline medium prepared *in situ* by on-line mixing of the acidified FDNB reagent solution with NaOH solution ($c = 1.5 \times 10^{-2}$ *M*) containing 5.0×10^{-4} *M* cetyltrimethylammonium bromide (micellar catalyst), and then the pH is adjusted on-line (second coil, 50 cm) to the optimum pH for the electrode operation (pH 5.5; TISAB).

As kinetic methods of analysis can be performed using various approaches, i.e., initial slope, fixed time, and reciprocal time, the first two were tested by Apostolakis et al.[31] Data for typical calibration graphs for acetaminophen concentration showed that linearity was good and the analytical concentration range (1 to 50×10^{-4} *M*) was suitable for drug analysis. Common excipients (gelatin, cellulose acetate-hydrogen phthalate, lactose, polyethylene glycol, magnesium stearate, starch, sodium lauryl sulfate, sugar, glucose, etc.) do not interfere except for carboxypolymethylene (carbopol), which gave negative errors (this might be due to the formation of mixed micellar solutions or partial saturation of the surfactant micelles, changing their catalytic action). Acetaminophen in tablets and elixirs was determined with good accuracy and precision (1.8 to 3.6% relative standard deviation, $n = 3$).

B. Adiphenine Hydrochloride

$C_{20}H_{21}ClNO_2$ (MM = 347.9)



Therap. category: anticholinergic

Discussion and Comments

A polymeric membrane electrode selective for adipheninium cation was constructed and characterized by Issa et al.[36] The elec-

trode is based on the incorporation of the adipheninium-tetraphenylborate ion-pair complex in poly(vinyl chloride) matrix plasticized with dioctylphthalate. Five membrane compositions were tested and the best results were obtained with that containing 8.0% ion-pair complex, 49.5% DOP, and 42.5% PVC (electrode performances: linear range, 3.0×10^{-2} to 1.5×10^{-5} M; slope, 56.3 mV/decade; pH range, 2.2 to 7.5; isothermal coefficient, 0.55 mV/ °C; interferences: tetrabutylammonium bromide, cetyltrimethylammonium bromide). The standard addition method and potentiometric titration were used to determine adiphenine hydrochloride in pure solutions as well as in pharmaceutical preparations such as ampoules (relative standard deviation in the potentiometric titration was found to be 0.5% for pure solutions and 1.9% for Spasmo–Cibalgin ampoules).

C. Alcohols

Low relative molecular mass alcohols (Table 1) are used widely as solvents in different chemical and pharmaceutical industries, and some of them (ethanol, 2-propanol) are recognized as topical antiseptic agents as well.

TABLE 1
Alcohols Assayed by Membrane Sensors

Alcohol	Formula	MM
Ethanol	C_2H_6O	46.1
1-Propanol	C_3H_8O	60.1
2-Propanol	C_3H_8O	60.1
2-Butanol	$C_4H_{10}O$	74.1

It was shown by Chan et al.[37] that primary and secondary alcohols can be converted quantitatively to xanthate (dithiocarbonate) in a NaOH-CS_2 two-phase system under phase-transfer catalysis, i.e.,

$$R-OH + CS_2 + NaOH \rightarrow R-O-\underset{\underset{S}{\|}}{C}-S^-Na^+ \quad (11)$$

and subsequently, the resultant xanthate was determined by a PVC membrane xanthate selective electrode based on tetraheptylammonium *O*-ethyl xanthate. The electrode was preconditioned in potassium *O*-ethyl xanthate solution (10^{-1} M) for 24 h before use and exhibited a Nernstian response down to 10^{-4} M alcohol with an average slope of 57 mV/decade at 25°C.

The derivatization of alcohols *in situ* takes place in the presence of tetrabutylammonium hydrogen sulfate as phase-transfer catalyst, at room temperature within 2 to 3 h. Since the PVC membrane xanthate-selective electrode gave stable and constant e.m.f. readings over a wide pH range (4.5 to 11), pH 9 was selected for subsequent potential measurements.

D. Alkaloids

Since the previous review[10] and monograph[11] were published, more research has been done in the field of alkaloid determination with membrane electrodes.[38-45] Table 2 lists the alkaloids covered by the recent literature in the field of ion-selective electrodes.

Atropine, codeine, and quinine, as well as different tertiary amines, were determined based on their reaction with methyl iodide.[38] The produced quaternary ammonium iodide salts were potentiometrically titrated with silver nitrate solution using either the iodide or silver sulfide ion-selective electrode. It was found that the reaction with methyl iodide is quantitative at the boiling point of CH_3I (43 °C), and the time required for the reaction to be completed depends on the reactivity of the tertiary nitrogen atom, but in all cases it did not exceed 90 min. In addition, the excess of methyl iodide reagent was completely evaporated during this time. Since a relatively slow titration was required to detect the end point (due to incomplete ionization of the quaternary salt in water), a small amount of sodium bicarbonate was added before the titration to facilitate the ionization of the respective quaternary salt. The pH of the aqueous solution should not exceed 8.0 to prevent the reaction of silver ions after the equivalence point. It was found that atropine and codeine react with methyl io-

TABLE 2
Alkaloids Assayed by Membrane Sensors

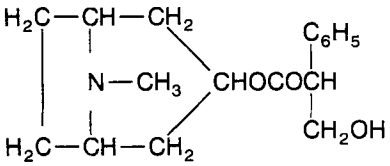
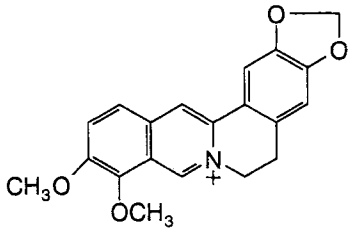
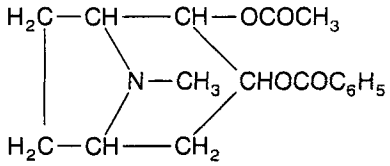
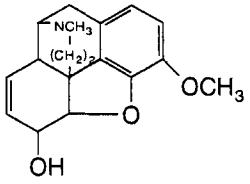
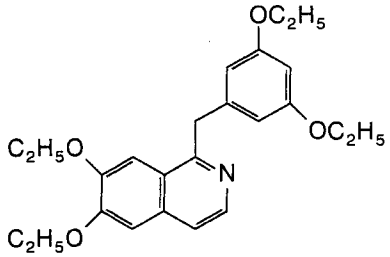
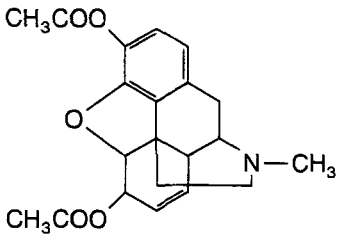
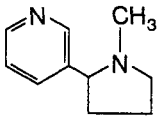
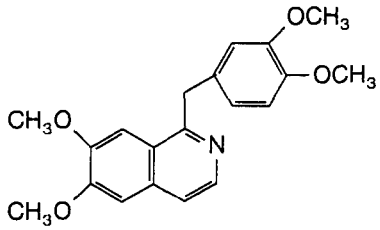
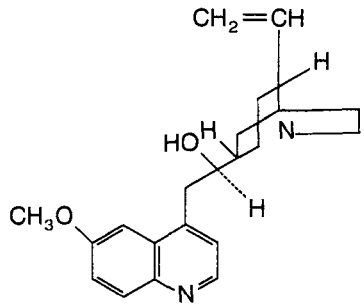
Alkaloid	Formula (MM)	Therap. category	Ref.
Atropine	$C_{17}H_{23}NO_3$(289.4) 	Anticholinergic	38
Berberine	$C_{20}H_{18}NO_4^+$(336.4) 	Bitter stomachic, antibacterial, antimalarial, antipyretic	39–41
Cocaine	$C_{17}H_{21}NO_4$ (303.4) 	Topical anesthetic (narcotic)	42
Codeine	$C_{18}H_{21}NO_3$ (291) 	Narcotic, analgesic, antitussive	38, 43
Ethaverine	$C_{24}H_{29}NO_4$ (395.5) 	Antispasmodic	45

TABLE 2 (continued)
Alkaloids Assayed by Membrane Sensors

Alkaloid	Formula (MM)	Therap. category	Ref.
Heroin (diacetylmorphine)	$C_{21}H_{23}NO_5$ (369.4) 	Narcotic, analgesic	44
Nicotine	$C_{10}H_{14}N_2$ (162.2) 	Ectoparasiticide	46
Papaverine	$C_{20}H_{21}NO_4$ (339.4) 	Smooth muscle relaxant, cerebral vasodilator	43, 45
Quinine	$C_{20}H_{24}N_2O_4$ (324.4) 	Antimalarial	38

dide in equimolar ratio to give an average recovery of 99.1%. However, quinine reacts with 3 mol of methyl iodide to form the corresponding methoxy-diquaternary ammonium salt (methylation at both N atoms as well as at —OH group).

The construction and analytical performances of berberine membrane electrodes were reported by Aubeck et al.,[39] Zhu,[40] and Liu et al.[41] When tetraphenylborate-berberine ion-pair complex was used as electroactive material in a PVC matrix, a detection limit of 10^{-8} M was attainable.[39] Chlorophyll (P 667), in a PVC membrane, showed good response to berberine in the concentration range 10^{-5} to 10^{-7} M with a slope of the electrode of 55.6 mV/decade at pH 5 to 11.

The construction of liquid membrane and PVC matrix-type cocaine ion-selective electrodes and their use for direct potentiometry and potentiometric titration of cocaine were recently described.[42] The ion-pair complexes of cocaine cation with reineckate and tetraphenylborate anions were either dissolved in nitrobenzene solvent or dispersed in a PVC matrix, with DOP or DBS plasticizer, and used as ion-exchange membranes. The electrochemical response characteristics of electrodes incorporating these types of membranes were evaluated with regard to the effect of pH, foreign basic compounds, temperature, and γ-radiation. The electrodes displayed a stable fast Nernstian response for 10^{-2} to 10^{-5} *M* cocainium cation, over the pH range 3 to 7, the lower limit of detection being 1 μg/ml. Determination as low as 20-μg/ml cocaine hydrochloride showed an average recovery of 98% and a mean standard deviation of $\pm 0.6\%$. The electrodes exhibited useful analytical characteristics for determining cocaine in some illicit powders and the results agreed fairly well with those obtained by gas–liquid chromatography.

The potentiometric response characteristics of a PVC membrane electrode for heroin based on its ion-pair complex with tetraphenylborate was also examined.[44] The membrane of the electrode was prepared with the composition of 2% ion-pair complex, 28% PVC, and 70% plasticizer (DOP or DBS). The calibration slopes were 55.2 ± 0.8 and 52.1 ± 0.9 mV/decade for DOP and DBS plasticized membranes, respectively (calibrations were made at constant pH and ionic strength using 0.1 *M* citrate buffer of pH 5 and the linear response was over the range 10^{-2} to 10^{-4} *M*). Illicit heroin powders may contain not only heroin, but also low levels of morphine, acetylcodeine, codeine, and some opium alkaloids.[44] The response of heroin-selective electrode to these interferents and the effects of some other alkaloids and basic compounds were investigated (selectivity coefficients lower than 0.1 were reported in all cases; separate solution method used). In six different illicit samples, the heroin content was found to be in the range of 10 to 36%, depending on the origin (standard addition potentiometry and potentiometric titration methods were used).

Ion-selective PVC membrane electrodes for the opium alkaloids papaverine and ethaverine were constructed and characterized by Eppelsheim et al.[45] The electrode membranes contain ion pairs of the alkaloids with the same TPB^- anion counterion (DBP as plasticizer). Detection limits of 2×10^{-6} *M* with near-Nernstian slopes were reported. The response of the papaverine electrode to ethaverine was about 10 times more sensitive than toward papaverine.

A comparative investigation of various selective electrodes with nitrobenzene liquid membranes as well as DBP-plasticized PVC in solutions of a number of physiologically active amines, including alkaloids such as codeine and papaverine, was conducted by Repin et al.[43] The advantages of electrodes with nitrobenzene membranes are related to their high electric conductivity and the stability of potential. On the other hand, the electrodes based on alkaloid-trioctylbenzene sulfonate ion pair in nitrobenzene (10^{-3} or 10^{-4} *M*) showed significant deviation from the linearity in concentrated solutions (10^{-2} *M* or higher).

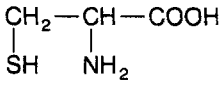
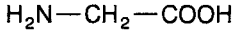
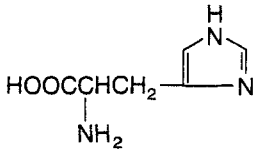
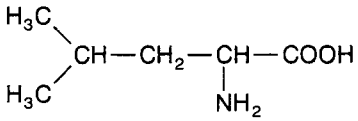
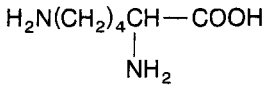
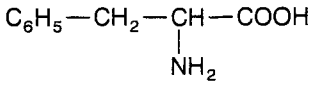
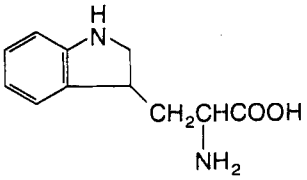
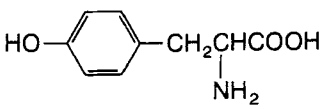
Five liquid membrane electrode systems responsive to the nicotinium cation were described by Hassan and Elnemma.[46] These electrodes are based on the use of ion-association complexes of the nicotinium cation with tetraphenylborate, 5-nitrobarbiturate, flavianate, reineckate, and picrolonate counter anions in nitrobenzene solvent as ion-exchange sites. The performance characteristics of these electrodes revealed fast, stable, and near-Nernstian responses for 10^{-2} to 10^{-5} *M* nicotine over the pH range 3.5 to 7.0. The direct potentiometric determination of 3 μg/ml to 1.6 mg/ml of nicotine in aqueous solutions showed an average recovery of 99.5% and a mean standard deviation of 1.2%. The electrodes were also used for monitoring the titration of nicotine with sodium tetraphenylborate, measuring the pK_a of nicotine and determining nicotine in the smoke from different cigarettes. The results compared favorably with those obtained by the standard gas chromatographic method.

E. Amino Acids

The amino acids listed in Table 3, all of them being of pharmacological interest, were recently determined with various membrane electrodes.[47-50]

As was pointed out in the previous review article[10], a kinetic-potentiometric method for the determination of amino acids, based on monitoring their reaction with 1-fluoro-2,4-dinitrobenzene (FDNB) using F^--selective electrode at pH 9.0 and 25°C, was described

TABLE 3
Amino Acids Assayed by Membrane Sensors

Amino acid	Formula (MM)	Therap. category	Ref.
Cysteine	$C_3H_7NO_2S$ (121.2) 	Has been used as detoxicant (vet.)	47, 49
Glycine	$C_2H_5NO_2$ (75.1) 	Nutrient	47, 48
Histidine	$C_6H_9N_3O_2$ (155.2) 	Elevated levels in physiological fluids are responsible for histidemia	47
Leucine	$C_6H_{13}NO_2$ (131.2) 	Nutrient	48
Lysine	$C_6H_{14}N_2O_2$ (146.1) 	Nutrient	47
Phenylalanine	$C_9H_{11}NO_2$ (165.2) 	Nutrient	47, 49
Tryptophan	$C_{11}H_{12}N_2O_2$ (204.2) 	Nutrient	47
Tyrosine	$C_9H_{11}NO_3$ (181.2) 	Nutrient	47, 49

by Athanasiou-Malaki and Koupparis.[32] The reaction of amino acids with FDNB is a well known example of a nucleophilic aromatic substitution reaction with the formation of an intermediate complex (see also Sections III.A, III.BB, and III.CC) and can be depicted by the following successive reactions:

$$R{-}NH_2 + (NO_2)_2C_6H_3F \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$$

$$(NO_2)_2C_6H_3^-(F)NH_2{}^+R \xrightarrow{k_2}$$

$$(NO_2)_2C_6H_3NHR + H^+ + F^- \quad (12)$$

Initial-slope and fixed-time (60-s) methods were used to construct calibration graphs, in most instances in the range 10^{-4} to 5×10^{-3} *M*. Later, the same authors[47] studied the effect of various surfactants on the above reaction. Micellar catalysis was provided by cetylmethylammonium bromide (CTABr), Aerosol AS, 22N and 501, and Triton X-405 even at levels below the critical micelle concentration. The micellar catalysis of CTABr in the reactions of 13 amino acids was described and correlation with the structure of the amino acids was analyzed. From the rate equation for bimolecular reactions and the Nernst equation for potentiometric-selective membrane electrodes, it was concluded that the initial slope of the potential/time curves can be used for calculation of reaction-rate constant:

$$(E/T)_0 = S'(1/[F^-]_0)K_{exp}[RHN_2]_0[FDNB]_0 \quad (13)$$

where S' is the slope of the E(mV) vs. ln[F^-] graph, which was evaluated by successive additions of sodium fluoride standards in a fixed volume of pH 9 borate buffer, $[F^-]_0$ is the initial fluoride concentration in the reacting mixture, and K_{exp} is the stoichiometric experimental rate constant of the second order reaction. The strongest catalysis was shown by CTABr. Its effect on the reaction of FDNB with various amino acids was examined in order to investigate any relationship between the extent of reaction acceleration and the molecular structure of each amino acid. Micellar catalysis appears for all the amino acids at the higher concentration of CTABr. At a concentration of 1.4×10^{-3} *M* CTABr, the relative acceleration is greater for tryptophan (1546%), aspartic acid (740%), tyrosine (706%), and phenylalanine (660%). The faster reaction shown by tryptophan, tyrosine, and phenylalanine may be attributed to the higher lipophilicity of their side chain (indole, phenol, and phenyl, respectively) and that of aspartic acid to the second carboxylate group.[47] The amino acids with slower reaction rates have side chains of higher polarity or are cationic (lysine). It was shown for cysteine that the reaction rate is high even without CTABr; FDNB reacts with the mercapto group instead of the amino group (pK_a = 10.78). Micelles shift the pK_a values and hence the degree of protonation of the reactive amino group. The lower acceleration (< 91%) found with sub-CMC levels of CTABr (9.2×10^{-5} *M* CTABr) must be attributed to the action of the organized assemblies formed below CMC.[47]

Campanella et al.[48] have constructed ion-selective liquid or PVC polymeric membrane sensors for alanine, leucine, and aspartic acid based on the use of benzyldimethylcetylammonium alaninate, leucinate, and aspartate exchangers, respectively. In all cases, short linear ranges and poor selectivities were observed.

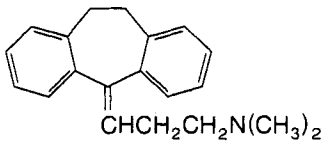
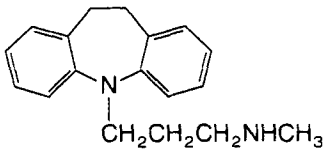
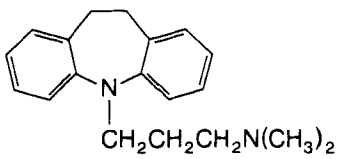
A method for the determination of specific amino acids (L-tyrosine and L-phenylalanine), based on the detection of amine formed by the enzymatic reaction of the respective amino acid with amino acid decarboxylase, using an amine-sensitive membrane electrode, was described by Katsu et al.[49] The corresponding amines, tyramine, and phenetylamine, respectively, were selectively detected by using a PVC-based membrane electrode containing sodium tetrakis(3,5-bis[trifluoromethyl] phenyl) borate as an ion exchanger and tricresyl phosphate as a solvent mediator. The detection limits for L-tyrosine and L-phenylalanine were 20 and 50 μM, respectively with a good linearity of the calibration curves up to ca. 10^{-3} *M* amine

(tyramine or phenetylamine) in 10 m*M* sodium acetate–acetic acid buffer solution of pH 5.5 (near-Nernstian slopes).

F. Amitriptyline and Related Compounds

The antidepressant drugs listed in Table 4 were recently determined by potentiometric membrane electrodes. The construction and general performance characteristics of potentiometric amitriptyline PVC membrane sensors, based on ion-pair complexes with tetraphenylstylbenyl borate and tetra(2-chlorophenyl) borate, respectively, were described by Bunaciu et al.[52] The complexes were obtained *in situ* by soaking the PVC membranes in 10^{-2} *M* amitriptyline hydrochloride. DOP and *o*-NPOE exhibited good behavior regarding response time and reproducibility of e.m.f. values of the electrodes. Both electrodes showed near-Nernstian responses over the range 1×10^{-2} to 7×10^{-6} *M* amitriptyline with a detection limit of about 5×10^{-6} *M* (reported electrode slope for both electrodes: 56.7 mV/decade). The electrodes proved useful in the determination of amitriptyline hydrochloride in pure drug substances and pharmaceutical preparations by using the potentiometric titration method (relative standard deviation < 2.0%). They were also applied for the determination of content uniformity and dissolution rate of sugar-coated amitriptyline tablets. The advantage of the electrode technique for measuring the dissolution rate of solid dosage forms is that the selective electrode can monitor continuously the concentration of the active ingredient in the standardized dissolution cells. The dissolution test was performed with a basket-stirrer USP-type apparatus operated at 50 rev/min in 250 ml of 0.1 *M* hydrochloric acid (simulated gastric fluid) (Figure 3). Figure 4 shows the dissolution profiles of sugar-coated amitriptyline tablets obtained by potentiometric method and by an UV spectrophotometric method. Both methods proved that the release of the active principle of the tablets in simulated gastric fluid follows the Langenbucher model,[54] i.e.,

TABLE 4
Amitriptyline and Related Compounds Assayed by Membrane Sensors

Compound	Formula (MM)	Ref.
Amitriptyline	$C_{20}H_{23}N$ (277.4) 	51, 52
Desipramine	$C_{18}H_{22}N_2$ (266.4) 	53
Imipramine	$C_{19}H_{24}N_2$ (280.4) 	53

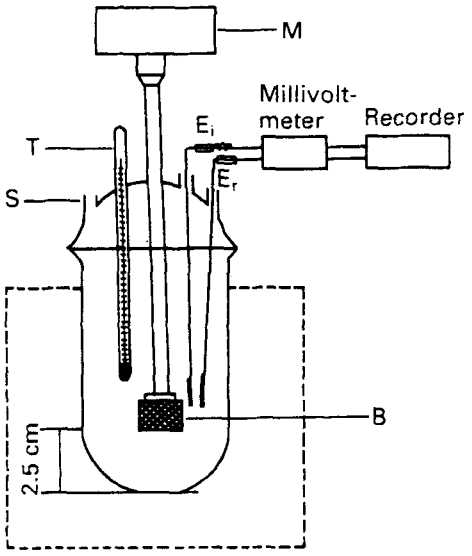


FIGURE 3. Modified USP basket-stirrer dissolution apparatus. T is the thermometer, S is the sampling device, E_i is the amitriptyline membrane electrode, and E_r is the SCE connected to the dissolution medium by a saturated KNO_3 agar-agar bridge. (Reproduced from Bunaciu, A.A. et al. *Analyst* **1991,** *116,* 239. With permission from The Royal Society of Chemistry.)

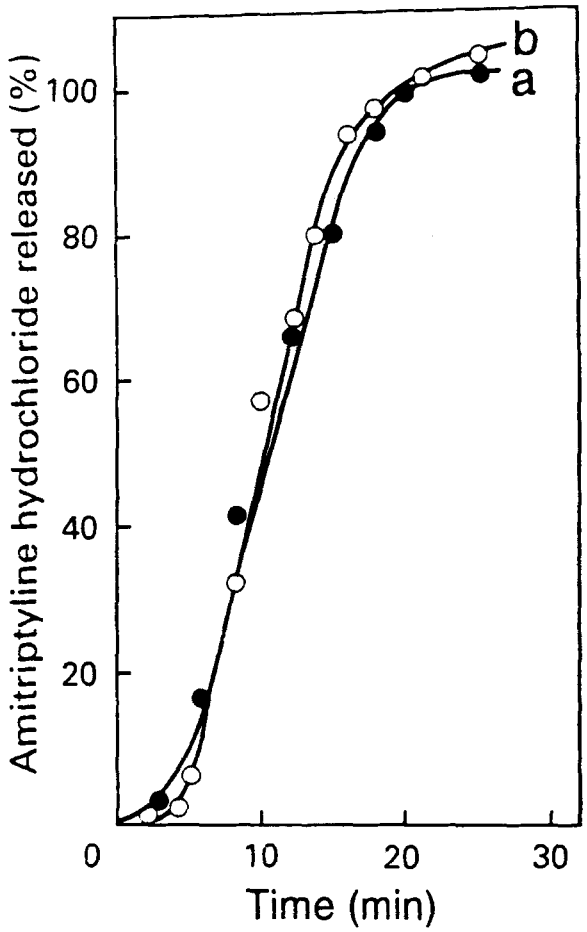


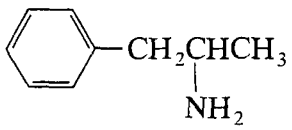
FIGURE 4. Dissolution profiles of sugar-coated amitriptyline tablets: a, potentiometric method; b, UV spectrophotometric method. (Reproduced from Bunaciu, A.A. et al. *Analyst* **1991,** *116,* 239. With permission from The Royal Society of Chemistry.)

the dissolution process involves two main steps: an initial step of about 4 min, while the coated layer is removed, followed by a rapid process of active principle dissolution.

Ion-selective electrodes sensitive to desipramine and imipramine, as well as to chlorpromazine (see Section III.MM), dicyclomine (see Section III.T), and propranolol (see Section III.PP) have been constructed using a modified poly(vinyl chloride) membrane which has ionic-exchange sites ($-SO_3H$) and which was cast using a solid polymeric plasticizer (Elvaloy 742, DuPont).[53] The polymeric-plasticized PVC membranes were prepared from the commercial PVC product which was conditioned with the respective drug (this procedure involves exchanging the hydrogen ion of $-SO_3H$ end charge groups with the drug cation). Both, desipramine and imipramine electrodes displayed Nernstian responses in the range 10^{-2} to 10^{6} *M* and 10^{-3} to 10^{-6} *M*, respectively (pH ranges: 2.0 to 6.5 for desiparaine electrode and 2.5 to 7.7 for imipramine electrode) and were used in pharmaceutical analysis.

G. Amphetamine

$C_9H_{13}N$ (MM = 135.2)



Therap. category: central stimulant

Discussion and Comments

Two novel electrode systems for selective determination of amphetamine, based on the use of dibenzo-18-crown-6 and dibenzo-24-crown-8 ionophores in dichloroethane as liq-

uid membranes were described by Hassan and Elnemma.[55] Both electrodes exhibited stable near-Nernstian responses over the range of 55 to 58 mV/decade. The working pH range is 3 to 7, the response time varied from 30 to 50 s, and the lower limit of detection was about 3 ppm. The selectivity toward amphetamine cation is relatively high relative to some inorganic cations and alkaloids such as brucine, cinchonine, and quinine as well as some amines structurally related to amphetamine (epinephrine, norepinephrine, ephedrine, phenylalanine, etc.). The DB18C6 membrane is 2 to 14 times more selective for amphetamine in the presence of foreign compounds than DB24C8-based electrodes. The shape of the cavity of the DB18C6 host is slightly flattened by the constraint of the benzo groups which allows a short host–guest contact distance and assists the $-NH_3^+$ site of amphetamine to anchor into the host cavity. The size of the DB18C6 cavity is suitable to fit the amphetamine–NH_3^+ group, leading to the formation of the relatively more stable complex.[55] Determination of 5 μg/ml to 4 mg/ml of amphetamine sulfate using the standard addition method showed an average recovery of 98.7% and a mean standard deviation of 1.7%.

Amphetamine and over 20 other primary and secondary amines were determined by a kinetic-potentiometric method, based on monitoring their reaction with 1-fluoro-2,4-dinitrobenzene (FDNB) by using a fluoride-selective electrode at pH 9 and 25°C[56] (see also Reference 32). Initial-slope and fixed-time (60 to 180-s) methods were used to construct calibration graphs in the range 10^{-4} to 10^{-3} *M*. The precision of the measurements was good for kinetic-potentiometric determinations with a relative standard deviation of 1.7%. The total measurement time for both kinetic methods ranged from 2 to 3 min, depending on the reaction rate.

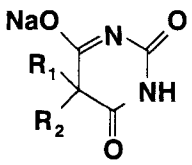
H. Barbiturate Drugs

The two barbiturate drugs (compounds with very well known hypnotic activity) listed in Table 5 were recently assayed by flow-through electrodes.[57,58]

The construction, assessment, and pharmaceutical applications of a tubular potentiometric detector sensitive to 5,5-diethylbarbiturate anion, based on quaternary ammonium salt (tetraoctylammonium 5,5-diethylbarbiturate) dissolved in *o*-nitrophenyl octyl ether (*o*-NPOE) immobilized in PVC, were described by Lima et al.[57] The tubular electrodes gave a linear response limit of ca. 3×10^{-4} *M*, a slope of 60 mV/decade, and a detection limit of 1×10^{-4} *M* (these values are slightly lower than those given by conventional-shaped electrodes). In the pH range 9 to 11.5, the potential of tubular detector is almost independent of pH and unaffected by the 5,5-diethylbarbiturate concentration.

A double-channel flow injection manifold (Figure 5) was used for analyses of pharmaceutical formulations. One channel was used to introduce a 2×10^{-5} *M* 5,5-diethylbarbiturate solution to carry the sample plug to the confluence point, where a solution to adjust the ionic strength and pH was added

TABLE 5
Barbiturates Assayed by Membrane Sensors



Compound	R_1	R_2	Formula (MM)
Barbital	$-C_2H_5$	$-C_2H_5$	$C_8H_{11}N_2NaO_3$ (206.2)
Phenobarbital	$-C_2H_5$	$-C_6H_5$	$C_{12}H_{11}N_2NaO_3$ (254.2)

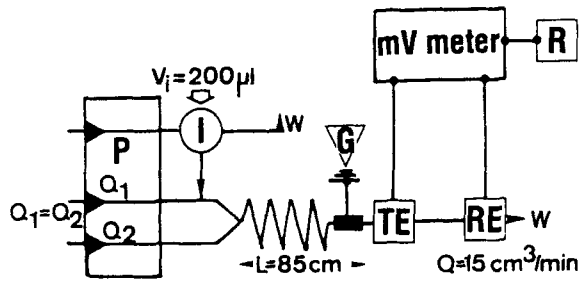


FIGURE 5. Flow injection manifold used in the determination of 5,5-diethylbarbiturate in pharmaceutical preparations: P is the peristaltic pump; GE is the grounding electrode, I is the injection valve, L is the dilution coil, TE is the tubular electrode (barbital selective), RE is the reference electrode, W is the waste, Q_1, Q_2 are flow rates; and V_i is the injection volume. (Reproduced from Lima, J.L.F.C.; Montenegro, M.C.B.S.M. *Anal. Chim. Acta* **1990**, *234*, 221. With permission from Elsevier Publishing Co.)

through a second channel. 5,5-Diethylbarbiturate was determined in commercially available tablets with a relative standard deviation of 0.7%, in good agreement with the BP reference method.

Tetraoctylammonium cation was also used as a counterion in the preparation of the ion-pair complex with 5-ethyl-5-phenylbarbiturate used to construct a polymeric flow-through electrode.[57] The good electrode performances (linear concentration range, 10^{-1} to 2×10^{-4} *M*; limit of detection, 1×10^{-4} *M*; slope, 58 mV/decade; pH range, 8.5 to 11) made possible its use for the determination of phenobarbital in pharmaceutical preparations (tablets and solutions).

I. Benzoic Acid

$C_7H_6O_2$ (MM = 122.1)

$C_6H_5—COOH$

Therap. category: antibacterial, antifungal

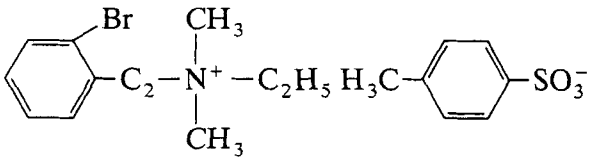
Discussion and Comments

Benzoate membrane electrodes based on the use of benzoate ion-pair complexes with tetraoctylammonium,[58] tridodecylmethylammonium,[60] and tetraheptylammonium[61] were recently described. The solid-state PVC flow-through benzoate electrode developed by Lima et al.[59] is based on a conductive support prepared with a mixture of a nonconductive epoxy and graphite. It was used as a potentiometric detector in flow injection analysis manifolds (benzoate determinations in medicinal syrups; recoveries varied between 94 and 106%).

Despite the potent interference by Cl^- ($-\log k = 1.3$), the benzoate electrode could be used for the determination of cholinesterase in blood serum at pH 7.4, phosphate buffer.[60] The method is based on the benzoate formation from benzoylcholine acetate (substrate) under enzymatic reaction. The cholinesterase activity was evaluated in terms of international units per liter of serum (IU/l) where 1 IU is defined as the amount that forms 1.0 µmol of benzoate per minute under established experimental conditions. It was observed that the rate of benzoate formation was proportional to the amount of serum added (5 to 20 µl).

J. Bretylium Tosilate

$C_{18}H_{24}BrNO_3S$ (MM = 414.4)



Therap. category: adrenergic blocker, antihypertensive

Discussion and Comments

Ion-selective poly(vinyl chloride) membrane electrodes and thick-film sensors for the determination of bretylium tosilate were recently described by Eppelsheim et al.[62] Ion-pair complexes of bretylium with the anionic counterions tungstosilicate, reineckate, tetraphenylborate, and dipicrylaminate were investigated as electroactive materials for the

electrode membranes. The membranes containing the bretylium tungstosilicate complex showed the best properties with both types of transducers. The detection limits were determined to be 1×10^{-7} *M* for the macro electrode and 8×10^{-7} *M* for the thick-film sensors in 0.1 *M* Tris buffer at pH 7.4 (slopes, 60.7 and 40 mV/decade, respectively). The potentiometric selectivity coefficients toward structurally similar compounds such as ephedrine, dopamine, and ephinephrine ranged from $10^{-1.7}$ to $10^{-3.4}$. Response times of 5 to 10 s were observed in buffered solutions as well as in human serum samples containing bretylium.

K. Bupivacaine and Other Local Anesthetic Drugs

Many local anesthetics in clinical use (see Table 6) are basically tertiary amino compounds and are classified as ester type (procaine, tetracaine, benzocaine, oxybuprocaine, etc.) or amide type (lidocaine, dibucaine, mepivacaine, bupivacaine, etc.). These drugs exist in both positively charged and uncharged forms under normal *in vivo* conditions.[63] Both species apparently have different biological activities.

New membrane electrodes sensitive to anesthetic drugs were recently described and the respective references are cited in the last column of Table 6. The electroactive materials are based on the ion-pair formation between either tetraphenylborate or phosphotungstate ions and the corresponding local anesthetic cations in a plasticized PVC membrane[63,64,66] or ethylene vinyl-acetate copolymer.[65] Shoukry et al.[64,66] constructed classical polymeric membrane electrodes, while Satake et al.[63] prepared coated-wire electrodes. Most of them displayed linear responses with near-Nernstian slopes in ranges depending on the drug structure, but generally covering 10^{-2} to 10^{-4} *M*. The selectivity of the electrodes depends on the selectivity of ion exchange at the membrane–test solution interface, the mobilities of the respective cations in the membrane phase, and hydrophobic interactions between the anesthetic cation and the organic membrane. The dibucaine electrode was found to have the most significant selectivity toward other local anesthetics, and the selectivity coefficients decreased in the order tetracaine, bupivacaine > mepivacaine, lidocaine > procaine > benzocaine. The difference in the hydrophobic property of local anesthetic molecules seems to be responsible for the difference in the electrode potential and the selectivity coefficients among the anesthetics.[63] The distribution coefficients between 1-octanol and buffer solution (pH 7.4), which reflect the hydrophobicity of anesthetic molecules, are known to be 1.7, 221, 43, 21, and 346 for procaine, tetracaine, lidocaine, mepivacaine, and bupivacaine, respectively.[67] The hydrophobicity of anesthetic molecules was closely related to the binding affinity of anesthetics to protein. The binding affinity increased in the order procaine < lidocaine < mepivacaine < tetracaine < bupivacaine. The larger the distribution coefficient of the anesthetic, the higher the electrode potential and selectivity to other anesthetics.[63]

It was proved by both potentiometric titration and direct potentiometry that the anesthetic–drug membrane electrodes are useful indicators for the determination of the respective drugs in either pure bulk substances or in pharmaceutical preparations (standard deviation < 0.5% when sodium tetraphenylborate solution was used as titrant). This result is well documented in the theory of ion exchange.[11]

L. Cephalosporins

Only two cephalosporins (Table 7) were reported to be determined with membrane electrodes in the last period of time.[47,68] They react with quaternary ammonium compounds to form ion-pair complexes that are only slightly soluble in water and which may, therefore, be used as liquid ion exchangers in a membrane phase for a cephalosporin electrode. On this principle, Dumkiewicz[68] prepared a cephalothin selective electrode.

TABLE 6
Bupivacaine and Related Drugs Assayed by Membrane Sensors

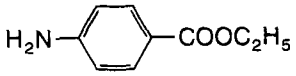
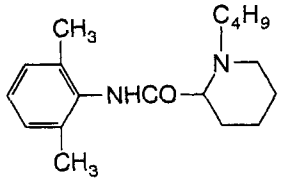
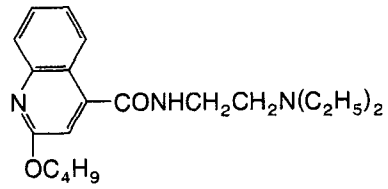
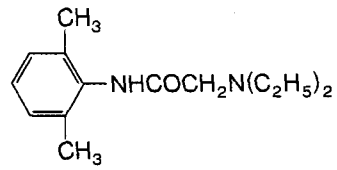
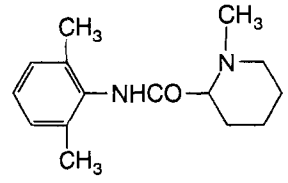
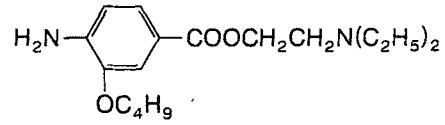
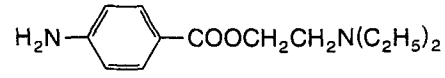
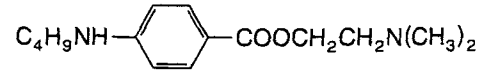
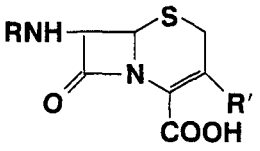
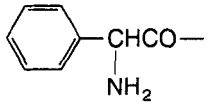
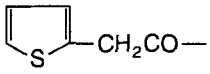
Drug substance	Formula (MM)	Ref.
Benzocaine	$C_9H_{11}NO_2$ (165.2) 	63, 64
Bupivacaine	$C_8H_{28}N_2O$ (288.4) 	63, 66
Dibucaine	$C_{20}H_{29}N_3O_2$ (341.4) 	63
Lidocaine	$C_{14}H_{22}N_2O$ (234.3) 	63–65
Mepivacaine	$C_{15}H_{22}N_2O$ (246.3) 	63
Oxybuprocaine	$C_{17}H_{28}N_2O_3$ (308.4) 	66
Procaine	$C_{13}H_{20}N_2O_2$ (236.3) 	63–65
Tetracaine	$C_{15}H_{24}N_2O_2$ (264.3) 	63, 65

TABLE 7
Cephalosporins Assayed by Membrane Sensors



Cephalosporin	Formula (MM)	R	R′
Cephalexin	$C_{16}H_{17}N_3O_4S$ (347.4)		$-CH_3$
Cephalothin	$C_{16}H_{16}N_2O_6S_2$ (396.4)		$-CH_2OCOCH_3$

The major component of the electrode is a "pseudoliquid potential-determining phase" (i.e., electroactive material) based on plasticized PVC which is in direct contact with an Ag/AgCl electrode. This is placed in a cylindrical PTFE container screwed onto the electrode frame. The electrode resistance depends on both the organic phase composition (e.g., addition of tributyl phosphate leads to a significant decrease in the resistance) and on the distance of the Ag/AgCl electrode from the sensor surface (at a distance of about 1 mm, the resistance is ca. 17 to 22 MΩ). This type of electrode has all the advantages of the coated-wire electrodes, but its lifetime is longer owing to the large volume of potential-determining phase which acts as a reservoir of the active substance. The Aliquat 336–cephalosporin ion-pair complex was used as electroactive material while dibutylphthalate was used as plasticizer solvent. The calibration graph, E(mV) vs. cephalothin concentration was linear in the range 10^{-1} to 10^{-4} M with a slope of 60.6 mV/decade. The cephalothin electrode selectivity for other cephalosporins (e.g., cephazolin, cephapirin, cephradine, etc.) is low. This is due to the structural similarity of the compounds and the slight effect of the substituents on the density of the charge collected on the carboxyl group.[68] Cephalothin has an acetoxymethyl group at position 3 of the dihydrothiazine system and a 2-thienylmethyl substituent on the β-lactam ring. These substituents, via an induction effect, cause a decrease of the charge density on the carboxyl group, which in turn causes a weakening of the interaction between the cephalothin ion and the liquid ion exchanger. Of those cephalosporins examined by Dumkiewicz,[68] cephalothin is characterized by having the strongest affinity for the ion exchanger. Nitrate and cloxacillin ions have a greater affinity for the ion exchanger than the cephalosporin ions, as shown by selectivity coefficients greater than unity. But these do not limit the applicability of the cephalothin electrode because penicillins are very rarely used in mixtures. Cephalothin, in the concentration range 43 to 436.5 mg/l, was determined by standard addition method with an accuracy comparable to that of the iodometric method recommended by BP.

Athanasiou-Malaki and Koupparis[47] described a detailed kinetic-potentiometric study based on the property of 1-fluoro-2,4-dinitrobenzene (FDNB, Sanger reagent) to undergo aromatic nucleophilic substitution by amines (including cephalexin) to give dinitrophenylamines and F^-. The last one was monitored by a fluoride-selective electrode. A micelle-catalyzed kinetic-potentiometric method was described and evaluated by drug assays in commercial formulations.[47] Either the initial-slope or the fixed-time method can be used for construction of calibration graphs. Excellent linearity and good precision (relative standard deviation < 2.0%) were ob-

tained with both methods. Detection limits of 1.4×10^{-4} *M* and 2.0×10^{-4} *M* were reported for cephalexin determination by initial-slope and fixed-time method, respectively (cetyltrimethylammonium bromide [CTABr] used as micellar catalyst).

M. Chlorobutane

C_4H_9Cl (MM = 92.6)

Therap. category: anthelmintic

Discussion and Comments

Chlorobutane and other alkylhalides were determined, via *in situ* generation of *S*-alkylisothiouronium salts, by a PVC membrane electrode based on *S*-alkylisothiouronium tetraphenylborate as electroactive material.[69] The reaction takes place in 95% ethanol in the presence of an excess of thiourea by means of a bimolecular nucleophilic substitution:

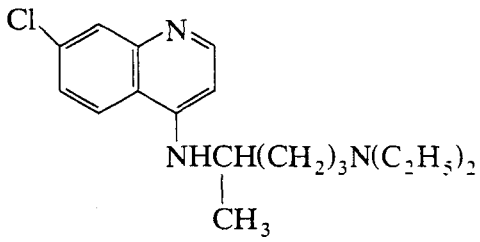
$$R{-}X + H_2N{-}\underset{\underset{S}{\|}}{C}{-}NH_2 \xrightarrow{\text{ethanol (95\%)}} R{-}S{-}\underset{\underset{{}^{+}NH_2X^{-}}{\|}}{C}{-}NH_2 \qquad (14)$$

where X^- is Br^- or I^-. For the less reactive halide such as chlorobutane, it was first necessary to convert these compounds into the corresponding alkyl iodides by refluxing overnight with an excess of sodium iodide in 95% ethanol. The crude iodo compound, without purification, was then converted into the *S*-alkylisothiouronium salt by treatment with an excess of thiourea (the mixture was refluxed for 150 min).

S-alkylisothiouronium selective electrode (DOP as plasticizer) exhibited Nernstian response in the range 1×10^{-1} to 1.6×10^{-4} *M* with an average cationic slope of 58.8 mV/decade. The electrode has a reasonably wide working pH range (6.5 to 8.5), fast dynamic response time (30 s to 2 min), stable response for at least 2 months, and high selectivity for *S*-alkylisothiouronium ion in the presence of many inorganic and organic ions. The average recovery for 1-iodobutane and 1-bromobutane of 98.1% (mean standard deviation of 0.88%) demonstrated that the method is quantitative and applicable for such compounds). For the less reactive alkyl halide, 1-chlorobutane, the two-step derivatization reaction is more prone to side reactions, such as elimination reactions. The recovery for this derivative was only 86.6%. However, the excellent precision observed for this electrode method rendered the determination of this less reactive halide equally feasible.[69] The determination of chlorobutane using the same calibration graph, adjusting the amount of it by a factor of 0.866, will give the equivalent amount of halide in the sample.

N. Chloroquine

$C_{18}H_{26}ClN_3$ (MM = 319.9)



Therap. category: antimalarial, antiamebic

Discussion and Comments

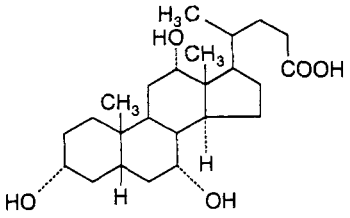
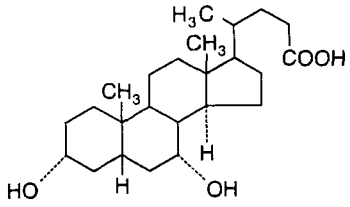
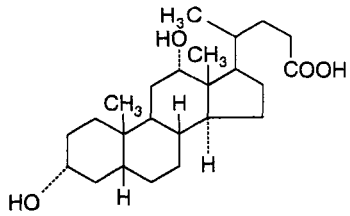
Two types of PVC matrix membrane electrode responsive to the chloroquine drug have been recently constructed, electrochemically evaluated, compared, and used in pharmaceutical analysis.[70] The first type is a classic PVC model with a chloroquine-tetraphenylborate sensor; the second type is a coated silver disk without internal filling solu-

tion. Both electrode types exhibited rapid linear response to logarithmic concentration of diprotonated chloroquine cation in the 10^{-1} to 10^{-5} *M* range with calibration slopes of 28 to 30 mV/decade over the pH range 1.8 to 6.2. These electrodes were sensitive enough to permit determination of chloroquine phosphate at concentrations as low as 5 μg/ml with good accuracy and precision. Determination of chloroquine in various pharmaceutical preparations using direct potentiometry and potentiometric titration with NaTPB gave an average recovery of 98.8% of the nominal values (sd = 0.5%). The second type electrode was also incorporated in a flow-through potentiometric cell for FIA. Results obtained for the determination of chloroquine in some pharmaceutical preparations by FIA showed an average recovery of 98.7% (sd = 0.5%, n = 45), in good agreement with the official USP method. The electrode methods offer several advantages: beside the omission of the time-consuming extraction step in the USP method, the electrode methods are more precise, directly applicable to automated systems, and not liable to interferences by the active ingredients, excipients, and diluents commonly used in chloroquine drug formulations.[70]

O. Cholic Acids

The development of a prototype ion-selective microelectrode for cholic acids (bile salts; see Table 8) was described by Kunze and Lindenbaum.[71] The microelectrodes demonstrated near-Nernstian response with sodium deoxycholate in Tris (0.2 *M*, pH 9.0, 15 to 30°C), Hepes (0.13 *M*, pH 7.5), and

TABLE 8
Cholic Acids Assayed by Membrane Sensors

Cholic acid	Formula (MM)
Cholic	$C_{24}H_{40}O_5$ (408.6) 
Chenodeoxycholic	$C_{24}H_{40}O_4$ (392.6) 
Deoxycholic	$C_{24}H_{40}O_4$ (392.6) 

bicarbonate (0.1 *M*, pH 7.5) buffers. They sensed also cholate, chenodeoxycholate and taurochenodeoxycholate ions, with slopes of 42 ± 1.3, 72 ± 1.1, and 69 ± 1.7 mV/decade, respectively.

The microelectrodes were fabricated from borosilicate glass capillary tubing with tips measuring < 1 to 20 μm. The retention of hydrophobic membrane solution (hexadecyltributylammonium deoxycholate complex in 1-octanol mixed with a THF solution of PVC and sebacic acid dioctyl ester) was enhanced by silanizing the inverted electrode bodies over dichlorodimethyl silane. The height of the organic PVC solution in the glass electrode body was 3 ± 1 mm. When this solution turned slightly opaque, an aqueous solution containing sodium deoxycholate (0.1 *M*) and NaCl (100 m*M*) was backfilled on the top. The bile salt-selective microelectrode had little selectivity among the compounds listed in Table 8, but was highly selective for bile salts over the inorganic cations, chloride and bicarbonate. The results of the selectivity studies, using the separate solutions method, showed that the responses of the electrode for deoxycholate, chenodeoxycholate, and taurochenodeoxycholate were practically indistinguishable whilst the response for sodium cholate was obviously lower.[71] This type of ion-selective microelectrode may show promise as a useful tool for the determination of intracellular bile salt activity.

P. Choline and Its Esters

Choline and its esters (Table 9) were recently determined with membrane selective electrodes.[72-74]

A choline sensitive membrane electrode based on a PVC membrane was constructed by using potassium-tetrakis-(4-chlorophenyl)-borate and *o*-NPOE as plasticizer.[72] It responded to choline down to 1×10^{-5} *M* (slope, 57 mV/decade) even in the presence of sodium deoxycholate (activator used in the enzymatic hydrolysis of phosphatidylcholine with phospholipase D). By using this electrode, choline formed by the enzymatic hydrolysis was successfully measured. The method was applied to the determination of phosphatidylcholine in serum, which is routinely determined spectrophotometrically by using three enzymes in sequence, i.e., phospholipase D, choline oxidase, and peroxidase. In comparison, the method proposed by Katsu et al.[72] uses only one enzyme, and thus the cost of routine analysis is less.

A new type of microsensor for acetylcholine based on acetylcholinesterase (AChE) immobilized on a coated-wire pH electrode was constructed by Taguchi et al.[73] AChE catalyzes the hydrolysis of acetylcholine; the hydrogen ion concentration thus increases and is monitored by the H^+-selective coated-wire electrode. AChE was covalently immobilized on the BSA-modified hydrogen ion-selective coated electrode

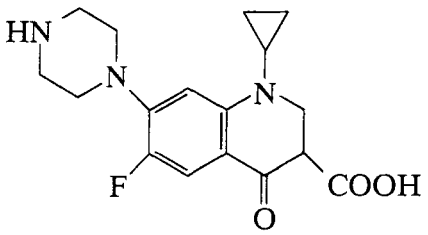
TABLE 9
Choline and Its Esters Assayed by Membrane Sensors

Compound	Formula (MM)	Therap. category
Choline chloride	$C_5H_{14}ClNO$ (139.6) $HOCH_2CH_2N^+(CH_3)_3Cl^-$	Parasympathomimetic
Acetylcholine bromide	$C_7H_{16}BrNO_2$ (226.1) $CH_3COOCH_2CH_2N^+(CH_3)_3Br^-$	Parasympathomimetic
Phosphatidylcholine	A mixture of the diglycerides of stearic, palmitic, and oleic acids linked to the choline ester of phosphoric acid	Lipotropic

(TDDA-based) with 1 mg/ml acetylcholinesterase solution in pH 7 phosphate buffer. Acetylcholine could be determined in the range 0.1 to 10 m*M* with response times of 3 to 10 min. pH changes within the range 6 to 8 had little effect on the determination; precise adjustment of the pH of the sample solution was unnecessary. Such long response times are due to the excessive thickness of the enzyme membrane in contact with the pH indicator electrode. The technique of enzyme immobilization that was described by Kumaran et al.[74] resulted in the formation of extremely thin films of reticulated enzyme (about 1 to 2 μm as shown by scanning electron microscopy). The immobilization, when done over glass electrodes, resulted in response times on the order of 5 to 10 s for substrate concentrations which lie in the linear ranges of measure. The electrodes prepared by this technique also showed homogeneous networks of enzyme and of enzyme cross-linked with glutaraldehyde after the spray. Calibration curves for the enzyme electrodes showed a linear range for the AChE from about 0.2 to 2 m*M* with a super-Nernstian slope of 188 mV/decade. Reasons for such behavior can be attributed to (a) the partial dissociation of the products and their transport properties in the enzyme layer, (b) the pH of the test solution, (c) the concentration of the buffering salts present in solution, and (d) the diffusion coefficients of the various species.[74]

Q. Ciprofloxacin

$C_{17}H_{18}FN_3O_3$ (MM = 331.4)



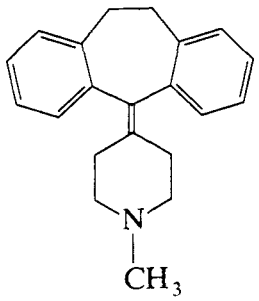
Therap. category: antibacterial

Discussion and Comments

A potentiometric sensor system characterized by a membrane based on a molecular dispersion of certain 4-quinolones and DOP as plasticizing solvent mediator in an inert PVC support was assessed for selectivity toward ciprofloxacin ions.[75] The electrodes which respond to ciprofloxacin ions have been prepared by coating a silver wire conductor with the PVC-based film. The preferred system with norfloxacin in the membrane showed a linear response with a near-Nernstian slope of 54 to 62 mV/decade of ciprofloxacin concentration over the range 10^{-4} to 10^{-2} *M*. The electrode exhibited good selectivity with respect to inorganic ions of biological interest, but did not show high selectivity to other investigated species (e.g., penicillins, cephalosporins, etc.). Ciprofloxacin could be determined with good results in its pure solutions and in some pharmaceutical preparations (tablets) using the standard addition technique (relative standard deviation = 0.16%, $n = 9$).

R. Cyproheptadine

$C_{21}H_{21}N$ (MM = 287.4)



Therap. category: antihistaminic, antipruritic, appetite stimulant

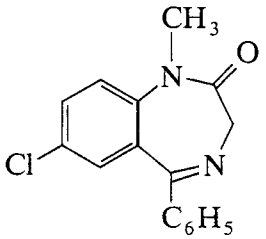
Discussion and Comments

A new cyproheptadine ion-selective membrane electrode based on the ion-pair complex with tetraphenylborate in PVC

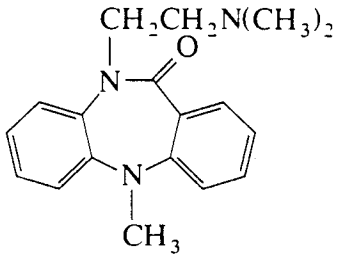
(DOP as plasticizer) was recently prepared and its performance characteristics studied in detail.[76] The electrode exhibited a linear response with a near-Nernstian slope over a relatively wide range of concentration (1×10^{-2} to 7.9×10^{-5} *M*). Up to 21 days of continuous soaking, the calibration graph slope was constant at 57.1 mV/decade, at 25°C; then it decreased as the time of soaking increased and reached 32.9 mV/decade after 37 days. The change in pH did not affect the electrode performance within the range 2 to 6. The electrode showed very good selectivity for cyproheptadine hydrochloride with respect to a large number of inorganic and organic cations. The standard addition method and potentiometric titration were used to determine cyproheptadine hydrochloride in pure solutions and in pharmaceutical preparations.

S. Diazepam and Dibenzepin

$C_{16}H_{13}ClN_2O$ (284.8)



$C_{18}H_{21}N_3O$ (295.4)



Therap. category: minor tranquilizers

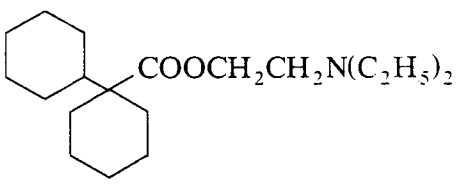
Discussion and Comments

Diazepam ion-selective electrodes with different electroactive materials in PVC matrix (DBP as plasticizer) were investigated by Nie et al.[77] in order to compare their electrode performances. The results obtained with ion-pair complexes of diazepam with tetraphenylborate, dipicrylaminate, heteropolyacid anions, and halogeno-metal complex acid anions showed that most of the trial electrodes presented nearly Nernstian cationic response over approximately 3 orders of magnitude in concentration, and the linearity ranges extended down to about 10^{-5} *M* (pH range 1.9 to 2.8). All the PVC membrane electrodes showed fast response (< 20 s in 10^{-2} to 10^{-5} *M* solutions) and good reproducibility of potential measurements (sd < 0.6 mV) and a lifetime of about 3 months. Among the foreign substances tested as interferents, only propantheline, berberine, chlordiazepoxide, chlorpheniramine, glycopyrolate, tetramisole, and tetrabutylammonium are likely to cause interference. However, these substances are rarely formulated in combination with diazepam.[77] Determination of diazepam by direct potentiometric method was performed with a mean recovery of 99.4% and a standard deviation of 2.3%. Diazepam also could be determined by potentiometric titration with sodium tetraphenylborate using the diazepam ion-selective electrode as indicator electrode (mean recovery, 99.4%; sd = 0.9%).

By coating a graphite rod with a film of PVC plasticized with a mixture of DOP-nitrobenzene and incorporated by dibenzepin-phosphotungstate ion-pair complex, a dibenzepin membrane electrode was obtained.[78] It responds to dibenzepin cation in the linear concentration range 10^{-3} to 2×10^{-6} *M* with a 53 mV/decade slope (pH range 5.1 to 6.9).

T. Dicyclomine

$C_{19}H_{35}NO_2$ (MM = 309.4)



Therap. category: anticholinergic

Discussion and Comments

A dicyclomine polymeric membrane electrode based on the use of PVC with $-SO_3H$ end groups and Elvaloy 742 (DuPont) plasticizer was described by Takisawa et al.[53] Its linear response range was 2×10^{-3} to 2×10^{-5} M with 56.1 ± 0.5 mV/decade slope (pH range 2 to 6.5). Apart from its potential application in pharmaceutical analysis, this electrode could also be used to investigate the interaction of dicyclomine with α- and β-cyclodextrins. The reasons for the use of cyclodextrin in such complexation studies were twofold: First, these compounds are known to inhibit the side effect of the drug; second, as cyclodextrins have been used as neutral carriers in membrane electrodes, data on complexation coefficients may give clues on how to improve the selectivity of these devices.[53] In general, a drug which is in solution with cyclodextrin is expected to form an inclusion compound, involving 1:1 complex (drug to cyclodextrin). Using the e.m.f. data (E[mV] vs. drug concentration with and without cyclodextrin) it was possible to determine the complexation constant, K, using the classical Schatchard equation in the form:

$$C/m_1 = K - KC \qquad (15)$$

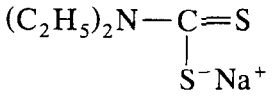
where C is the concentration of drug complexed with cyclodextrin per total concentration of cyclodextrin and m_1 is the drug monomer concentration at each total concentration of drug C_{tot} for which the measurements were taken. The plots of C/m_1 vs. C for the data involving dicyclomine hydrochloride binding to α- or β-cyclodextrin showed that binding to α-cyclodextrin is ca. 100 times less than that of β-cyclodextrin. The Bensi–Hildebrand plot in the form $1/C$ vs. $1/m_1$ [Eq. 16] was used by Takisawa et al.[53]:

$$1/C = (1/Km_1) + 1 \qquad (16)$$

The linearity and intercept of this plot confirmed the 1:1 stoichiometry of the complex and the equilibrium constants of $(2.8 \pm 0.3) \times 10^2$ and $(9.5 \pm 0.9) \times 10^4$ were obtained for α-cyclodextrin and β-cyclodextrin, respectively. A modified β-cyclodextrin may be used as a neutral carrier in the membrane in order to develop selective drug membrane electrodes.

U. Diethyldithiocarbamate (Sodium Salt)

$C_5H_{10}NNaS_2$ (MM = 171.3)



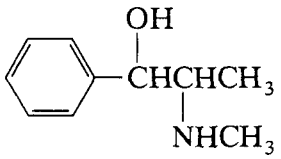
Therap. category: chelating agent, experimental in Wilson's disease

Discussion and Comments

The preparation and usability of a sulfide ion-selective microelectrode, obtained by chemical pretreatment of silver wire with Hg^{2+} and sulfidization in 0.1 M alkaline solution of Na_2S for 15 min was described.[79] The electrode, which is suitable for direct potentiometric determination of sulfide in alkali solutions of concentrations down to 4×10^{-7} M, could also be used for the potentiometric measurement of various thio compounds in alkali and neutral media. The electrode displayed a near-Nernstian response to diethyldithiocarbamate anion (in 0.1 M NaOH) in the range 10^{-2} to 5×10^{-6} M (slope, 56.5 mV/decade; detection limit, 1.7×10^{-7} M; response time < 2 min) and could be used for its determination.

V. Ephedrine

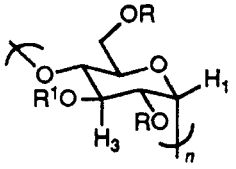
$C_{10}H_{15}NO$ (MM = 165.2)



Therap. category: sympathomimetic (L form as adrenergic bronchodilator)

Discussion and Comments

The development of a potentiometric ion-selective electrode that can be calibrated to read the enantiomeric purity of a chiral analyte is an attractive target. Recent work performed by Bates et al.[80,81] showed that a lipophilic peroctylated α-cyclodextrin could be used in a potentiometric ion-selective electrode to measure the enantiomeric purity of ephedrine in the presence of serum cations. Octylation of α-cyclodextrin with $C_8H_{17}Br$ in NaOH–Me_2SO at 20°C yielded the 2,6-di-*O*-octyl-α-cyclodextrin 1a (Figure 6). Further alkylation was achieved under more forcing conditions (NaH, THF, 60°C, 4 days) to give 2,3,6-tri-*O*-octyl derivative 1b. Peroctylation of β-cyclodextrin was performed in a similar manner yielding 1c and 1d. Using 1 to 2 mol% 1b, an electroactive membrane was obtained (32.8% PVC, 0.4% KTpClPB) using either bis(butylpentyl) adipate (BBPA) or *o*-NPOE as plasticizer. With BBPA plasticizer using 1 m*M* NH_4Cl inner filling solution, the electrode gave a Nernstian response to (+)ephedrinium hydrochloride with a limit of detection of $10^{-6.5}$ *M*. This sensitivity was only slightly affected by the presence of serum levels of Na^+, K^+, and Ca^{2+}. With (−)ephedrinium hydrochloride, a reduced slope was observed (50 mV/decade) and the limit of detection was $10^{-6.6}$ *M*. The "bias" potential of the two electrodes [one conditioned in 0.1 *M* (+)ephedrinium hydrochloride; the other with the (−)enantiomer] was measured in a cell with no liquid junctions, giving a value of 24.5 ± 0.5 mV with BBPA as plasticizer, at room temperature, constant over 4 h. This e.m.f. difference may be related to a free energy difference of 2.4 ± 0.05 kJ/mol for formation of the diastereoisomeric cyclodextrin complexes in the membrane sensor.[80] Using *o*-NPOE as plasticizer, the electrode response to (+) ephedrine hydrochloride was near Nernstian with a detection limit of $10^{-5.25}$, but with (−)ephedrine hydrochloride, a reversal of slope was observed in solutions more dilute than $10^{-2.8}$ *M* (a likely explanation of this effect is related to the competitive binding of the plasticizer by the peroctyl-α-cyclodextrin). Using solutions of predetermined enantiomeric purity, the electrode response was measured and the electrode could be calibrated (and used over a period of at least 3 months) to measure directly the enantiomeric purity of the ephedrine salt.

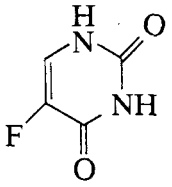


1a; R = C_8H_{17}, R^1 = H, *n* = 6
b; R = R^1 = C_8H_{17}, *n* = 6
c; R = C_8H_{17}, R^1 = H, *n* = 7
d; R = R^1 = C_8H_{17}, *n* = 7

FIGURE 6. Peroctylated cyclodextrins used for a chiral potentiometric ephedrine sensor. (Reproduced from Bates, P.B. et al. *J. Chem. Soc. Chem. Commun.* **1992,** 153. With permission from The Royal Society of Chemistry.)

W. Fluorouracil

$C_4H_3FN_2O_2$ (MM = 130.1)



Therap. category: antineoplastic agent

Discussion and Comments

5-Fluorouracil and other substituted uracils were potentiometrically titrated with 0.01 *M* ethanolic $AgNO_3$ solution by using a silver sulfide ion-selective membrane electrode as indicator electrode and a partially nonaqueous borate buffer solution as reaction medium (1:1 borate buffer:ethanol). A complex fluouracil:Ag (1:2 ratio) was formed

during titration.[82] The potential jump around the equivalence point was ca. 130 mV.

X. Gentamicin and Related Antibiotics

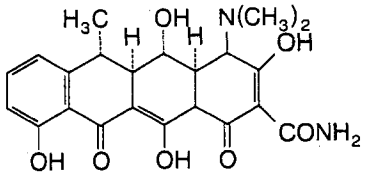
Antibiotics as listed in Table 10 were recently assayed by membrane electrodes techniques.

The kinetic-potentiometric method described for the determination of various primary and secondary amines, based on monitoring their reaction with 1-fluoro-2,4-dinitrobenzene (FDNB) by using a fluoride-selective electrode at pH 9.0 and 25°C, is applicable for the determination of gentamicin and tobramycin as well[33] (Table 10). Initial-slope and fixed-time 60 to 180-s) methods were used to construct calibration graphs in the range 10^{-4} to 10^{-3} M. Both antibiotics were determined in commercial formulations with a precision and accuracy of 2 to 3% and the results were comparable with those obtained by the time-consuming official microbiological assay.

Liu and Wu[83] described Nafion-modified electrodes sensitive to doxycycline. They respond linearly to monoprotonated doxycycline (pH 0.9 to 2.2) in the concentration range $1 \times 10^{2.7}$ to $1 \times 10^{-5.3}$ M with detection limits of 2.5×10^{-6} M (graphite substrate) and 2.2×10^{-6} M (glassy carbon substrate). Both were successfully applied for doxycyline determination by direct potentiometry.

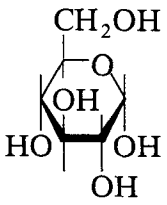
Erythromycin-sensitive electrodes with different electroactive materials in PVC membranes, both of conventional type and all solid-state, were described by Yao et al.[84] Electrode functions deteriorated in the order of silicotungstate > dicyclohexylnaphthalene sulfonate > tetraphenylborate > dipicrylaminate. The silicotungstate-type electrode (5 to 10 mM active material in PVC membrane plasticized with DBP) showed the best performances with respect to linear range, slope and detection limit (values of 10 to 0.02 mM, 59.4 ± 0.4 mV/decade, and 2 μM, respectively, were reported; pH range, 1.9 to 4.8). An electrode based on the same electroactive material with Pt- or Au-plated substrate exhibited nearly the same performance as the conventional electrode. Electrode selectivity toward quaternary ammonium cations was described in terms of induction and steric hindrance effect.[84] Erythromycin was determined by direct potentiometry with an erythromycin-silicotungstate electrode with an average recovery of 99.9% and a standard deviation of 0.6% (n = 6).

TABLE 10
Gentamicin and Related Antibiotics Assayed by Membrane Sensors

Antibiotic	Formula (MM)	Ref.
Gentamicin	Antibiotic complex produced by fermentation of micromonospora purpurea or micromonospora echiaospora	33
Doxycycline	$C_{22}H_{24}N_2O_{13}$ (444.4) 	83
Erythromycin	$C_{37}H_{67}NO_3$ (733.9)	84
Tobramycin	$C_{18}H_{37}N_5O_9$ (467.5)	33

Y. Glucose

$C_6H_{12}O_6$ (MM = 180.2)



Therap. category: fluid and nutrient replenisher

Discussion and Comments

A trinitrobenzenesulfonate (TNBS) ion-selective liquid membrane electrode[85] was used for the kinetic determination of glucose and some other carbohydrates.[86] The reaction of TNBS with an excess of carbohydrate in alkaline medium (0.3 *M* NaOH) must be carried out at 45 to 60°C, because at lower temperatures the reaction rate is low. The recorded graph (E vs. time) for the reaction of glucose with TNBS was linear (the reaction is first order with respect to TNBS). The reaction with TNBS was applied for the determination of invert after hydrolysis to glucose and fructose. The relative standard deviations for 4×10^{-3} *M* glucose and 8×10^{-3} *M* invert sugar were 2.0 and 1.1%, respectively ($n = 10$).

Z. Heparin

Highly sulfated dextrorotatory mucopolysaccharide composed of D-glucosamine and D-glucuronic acid residues. MM varies from 6,000 to 20,000 depending on source and method determination.

Therap. category: anticoagulant (sodium salt); potassium salt has antihyperlipidemic properties

Discussion and Comments

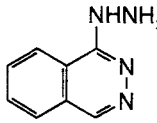
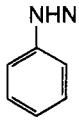
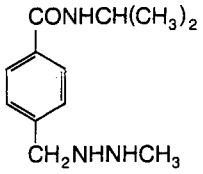
An interesting study on designing a heparin-responsive electrochemical sensor was recently performed by Meyerhoff and coworkers.[87] Their studies were focused on the use of a quaternary ammonium salt, tridodecylmethylammonium chloride (TDMAC) as the "heparin carrier" incorporated within the polymer membrane phase. Membranes formulated with a lower weight percent plasticizer (DOS) relative to the PVC content exhibited the greatest response to heparin. A membrane composition with 65% PVC, 33% DOS, and 1.4 to 2.0% TDMAC had optimum response to heparin. The electrode containing this membrane was able to detect low levels of heparin (0.1 to 1.0 units/ml) in solution even in the presence of 0.12 *M* Cl^-. Linear potentiometric response was also observed in undiluted human plasma samples at heparin concentrations between 1.0 and 9.8 units/ml. The reduced response in plasma vs. saline solution may be due to the binding of heparin to ondogenous proteins (e.g., antithrombin-III) and/or the inhibition of heparin extraction into the membrane phase by surface-adsorbed plasma proteins.[87]

Among many glycosaminoglycans tested as possible interferences in the electrode response (e.g., dermatan sulfate, chondroitin sulfate, hyaluronic acid), the heparin electrode was found to yield increased potentiometric response correlating to the degree of sulfate content of these compounds. The electrode displayed no measurable response to poly(vinyl sulfate), a highly sulfated polymer (62% sulfate content), nor to sulfated and nonsulfated glucosamine residues, which are the major monosaccharide building blocks of heparin.[87]

AA. Hydrazine Derivatives

A kinetic-potentiometric method was described for the determination of some hydrazines with biological activity[88] (see Table 11). The method is based on monitoring their reactions at 25°C and pH 9.0 [mixed borate

TABLE 11
Hydrazine Derivatives Assayed by Membrane Sensors

Hydrazine	Formula (MM)	Therap. category
Hydralazine	$C_8H_8N_4$ (160.2) 	Antihypertensive
Penylhydrazine	$C_6H_8N_2$ (108.1) 	Hemolytic
Procarbazine	$C_{12}H_{19}N_3O$ (221.3) 	Antineoplastic

(0.03 *M*)–fluoride (3×10^{-3} *M*)–*trans*-1,2-diamino-cyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (DCTA) (0.05 *M*) buffer] with 1-fluoro-2,4-dinitrobenzene (FDNB) by means of a fluoride-selective electrode.

The reaction of hydrazines with FDNB is a nucleophilic aromatic substitution with formation of an intermediate complex (see also Section III.E):

$$\mathrm{R{-}NH{-}NH_2} + (\mathrm{NO_2})_2\mathrm{C_6H_3F} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$$

$$(\mathrm{NO_2})_2\mathrm{C_6H_3^-(F)NH_2^+NHR} \xrightarrow{k_2}$$

$$(\mathrm{NO_2})_2\mathrm{C_6H_3NHNHR} + \mathrm{H^+} + \mathrm{F^-} \quad (17)$$

Assuming a steady state, the rate of F^- formation is described by

$$d[\mathrm{F^-}]/dt = [k_1k_2/(k_1 + k_2)] \times [\mathrm{RNHNH_2}]_t[\mathrm{FDNB}]_t = k_{\mathrm{exp}}[\mathrm{RNHNH_2}]_t[\mathrm{FDNB}]_t \quad (18)$$

where k_{exp} is the overall experimental second-order rate constant. By differentiation of the Nernst equation for the fluoride membrane electrode with respect to time, the following equation is obtained:

$$dE/dt = S'(1/[\mathrm{F^-}])(d[\mathrm{F^-}]/dt) \quad (19)$$

where S' is the slope of the calibration graph (E vs. $\ln[\mathrm{F^-}]$). Combining Eqs. 17 and 18, at the start of the reaction where the initial slope is measured, the following equation is obtained:

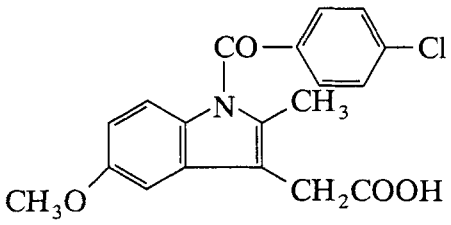
$$(\Delta E/\Delta t)_0 = S'(1/[\mathrm{F^-}])_0 k_{\mathrm{exp}}[\mathrm{RNHNH_2}]_0[\mathrm{FDNB}]_0 \quad (20)$$

which is the basis for the hydrazine determination method.[88] From Eq. 20, the initial slope of the reaction curve is linearly related to the analyte concentration (the reaction is the first order). The proposed method was successfully applied to the determination of hydrazine compounds in commercial formulations. Good precision (1 to 2% relative

standard deviation; three samples each measured three times) and good agreement with the reference methods were obtained. The excipients in the formulations analyzed had no effect on the determination.

BB. Indomethacin

$C_{19}H_{16}ClNO_4$ (MM = 357.8)



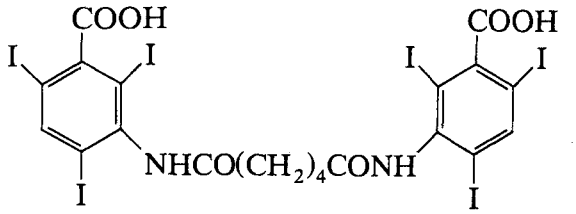
Therap. category: anti-inflammatory, antipyretic, analgesic

Discussion and Comments

The properties of an ion-selective electrode for indomethacin based on the bis(triphenylphosphoranilidene) ammonium-indomethacin ion-pair complex in a PVC membrane (*o*-NPOE as plasticizer) were described by Aubeck et al.[89] The detection limit found for indomethacin at pH 7.0 was 2×10^{-5} *M* (6.4 μg/ml) and the linear range was determined to be 5×10^{-5} to 10^{-3} *M*. The selectivity coefficients observed for organic anions of different lipophilicity were $10^{-1.1}$ for naproxenate, $10^{-0.75}$ for salicylate, and $< 10^{-4}$ for tartrate. A serious interference was observed for inorganic anions such as IO_4^-, ClO_4^-, and SCN^- with selectivity coefficients up to $10^{0.9}$. The electrode slope at pH 7.0 was 88 ± 1.5 mV/decade in both Tris-HCl and in sodium phosphate buffer. Measurements in more alkaline media of pH 9 and 11 showed an almost Nernstian slope of 58 mV/decade. This observation can be explained by reference to the pK_a value of indomethacin ($pK_a = 4.5$) at pH 7.0, when only partial dissociation of the carboxylic group occurs, and by the formation of dimers, which is typical of carboxylic acids.[89]

CC. Iodipamide

$C_{20}H_{14}I_6N_2O_6$ (MM = 1139.8)



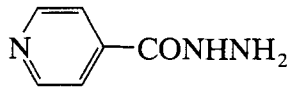
Therap. category: diagnostic aid (radiopaque medium)

Discussion and Comments

Conventional PVC membrane electrodes and membrane electrodes of all solid-state construction, selective to iodipamide, were prepared with different ion-association complexes as electroactive materials (e.g., cetyltrioctylammonium iodipamide, cetyltriphenylphosphonium iodipamide) and their electrode performances were reported by Yao et al.[90] Both the long-carbon-chain quaternary ammonium and quaternary phosphonium compounds yield good electrode performances (large linear concentration range; Nernstian slope, corresponding to a divalent anion; pH 9.4 to 12.0). No significant interference was observed by most of the substances tested including benzoate, glutamate, diatrizoate, and nicotinate. Results of the direct potentiometric assay of iodipamide using a calibration curve showed an average recovery of 98.9%. The electrode was applied for the determination of iodipamide meglumine injection and the analysis results compared well with USP method.

DD. Isoniazid

$C_6H_7N_3O$ (MM = 137.2)



Therap. category: antibacterial (tuberculostatic)

Discussion and Comments

The kinetic-potentiometric method described in Section III.AA was also applied for the determination of isoniazid.[88] Figure 7 shows the typical E(mV) vs. time curves of the FDNB-isoniazid reaction. The shape of the reaction curves suggests that the initial-rate and fixed-time methods can be used for construction of calibration graphs, i.e., $\Delta E/\Delta t$ vs. concentration (initial-rate method) or $(10^{\Delta E/S} - 1)$ vs. concentration (fixed-time method), where S is the fluoride electrode slope determined with buffered fluoride standard solutions. The very simple and selective determination of isoniazid in the presence of highly colored rifamycin is a particular advantage over the usual analytical procedures. Isoniazid could also be determined by a flow injection kinetic-potentiometric method, based on the same reaction with FDNB, but this time catalyzed by micelles of cetyltrimethylammonium bromide (see Section III.A and Reference 31).

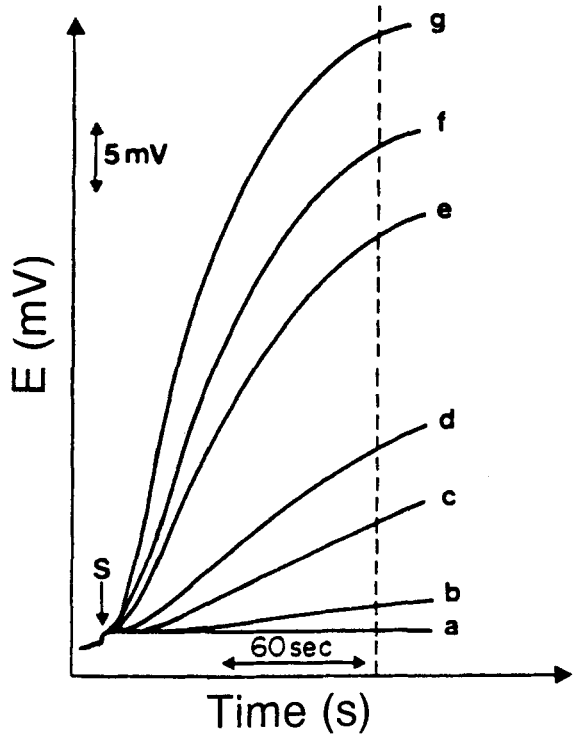
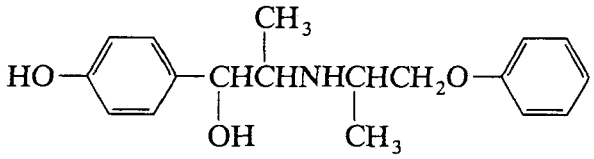


FIGURE 7. Typical reaction curves of the FDNB-isoniazid reaction for the calculation of the kinetic parameters and calibration graphs, at 25°C and pH 9.0 [FDNB] = 1.78×10^{-3} M; isoniazid concentrations: (a) blank, (b) 6.67×10^{-5} M, (c) 3.33×10^{-4} M, (d) 6.67×10^{-4} M, (e) 2.00×10^{-3} M, (f) 3.33×10^{-3} M, (g) 6.67×10^{-3} M. The dashed line shows the ΔE measurement in the fixed-time method. (Reproduced from Athanasiou-Malaki, E.; Koupparis, M.A. *Talanta* **1989**, *36*, 431. With permission from Pergamon Press).

EE. Isoxsuprine

$C_{18}H_{23}NO_3$ (MM = 301.4)



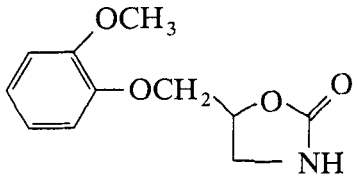
Therap. category: vasodilator

Discussion and Comments

The kinetic flow injection method described by Apostolakis et al.[31] is applicable to isoxsuprine, which is a phenolic drug. The method is based on monitoring the liberation of fluoride during the reaction of this drug with 1-fluoro-2,4-dinitrobenzene (FDNB). The reaction rate, catalyzed by cetylmethylammonium bromide, is monitored by a fluoride-selective electrode in a wall-jet configuration and is used to construct a calibration graph of antilog $(\Delta E/S) - 1$ vs. concentration, using the fixed-time approach (see also Figure 2). The linearity of the calibration graph was good and the analytical range (10^{-4} to 5×10^{-3} M) was suitable for drug analysis. The results were in good agreement with those given by reference methods.

FF. Mephenoxalone

$C_{11}H_{13}NO_4$ (MM = 223.2)



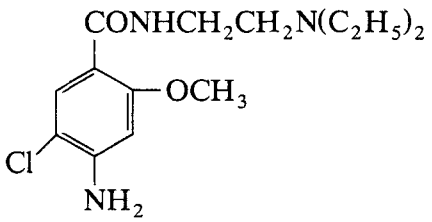
Therap. category: anxiolytic, skeletal muscle relaxant

Discussion and Comments

It was found that mephenoxalone decomposed in alkaline medium to yield three unidentified products (detected by TLC method) which could be precipitated with sodium tetraphenylborate solution. On this basis, an assay method for mephenoxalone was developed in which a simple potentiometric coated-wire electrode, prepared by coating an aluminum wire with a membrane formed by PVC plasticized with 2,4-dinitrophenyl-*n*-octyl ether, was used to monitor the ion-pair formation titration.[91] The hydrolysis of mephenoxalone was carried out with 1 *M* NaOH (boiling for 15 min). The protonized products, obtained after hydrolysis and acidification of the reaction mixture with 20% HCl to pH 3.0, were potentiometrically titrated with 0.04 *M* sodium tetraphenylborate. The method was applied for the determination of mephenoxalone in tablets and it compared well with both differential scanning calorimetry and UV spectrometry.

GG. Metoclopramide

$C_{14}H_{22}ClN_3O_2$ (MM = 299.8)



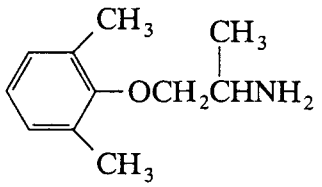
Therap. category: antiemetic

Discussion and Comments

A metoclopramide double-membrane ion-selective electrode, based on an internal conducting membrane of tetrabutylammonium bromide and an external membrane containing the ion-pair complex of metoclopramide with sodium tetraphenylborate has been prepared by Diaz et al.[92] Dibutylphthalate was used as plasticizer and PVC was used as an inert matrix. The internal membrane decreases the electrical resistance and gives good electrical contact. Its use improves the selectivity, reproducibility, and the stability of e.m.f. measurements. The composition of the active external membrane is of great importance in obtaining a Nernstian response. The obtained metoclopramide electrode exhibited a slope of 57.9 mV/decade at 25°C for a composition of ion-pair complex, DBP and PVC + THF solution 5% (m/v) in a ratio 35:10:55 by weight, respectively. The linear response of the electrode covered the range 10^{-1} to 10^{-5} *M* with a detection limit of 1×10^{-5} *M* metoclopramide (pH 3 to 7). The electrode was applied to the determination of metoclopramide in pure solutions and in a pharmaceutical product by direct potentiometry using the calibration graph and standard addition methods (standard deviations for the determination of metoclopramide tablets were 1.4% by the calibration graph method and 1.03% by the standard addition method, respectively.

HH. Mexiletine

$C_{11}H_{17}NO$ (MM = 179.3)



Therap. category: antiarrhythmic

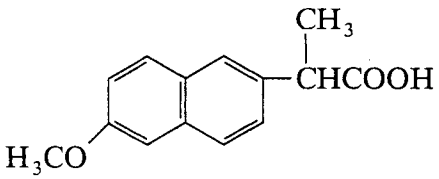
Discussion and Comments

PVC membrane electrodes prepared with mexiletine-tetraphenylborate, mexiletinereineckate, mexiletine-picrolonate, and mexiletinedipicrylamine ion-pair complexes, respectively, were studied for their analytical performances by Leng and Hu.[93] The best membrane electrode was that containing mexiletine-tetraphenylborate as the electroactive material and DOP as the solvent plasticizer. The linear response covered the

range from 10^{-1} to 1.5×10^{-5} M mexiletine with a slope of 57.6 mV/decade (pH range 2 to 8). The electrode showed fast response, good reproducibility, and stability. The direct potentiometry method was used to determine mexiletine in various samples with an average recovery of 99.3% and a standard deviation of 1.2%.

II. Naproxen

$C_{14}H_{14}O_3$ (MM = 230.3)



Therap. category: anti-inflammatory, analgesic, antipyretic

Discussion and Comments

A naproxenate-selective electrode with a liquid membrane consisting of a tetraheptylammonium-naproxenate ion-pair dissolved in p-nitrocumene was described by Valsani et al.[94] Various other bulky quaternary ammonium cations (e.g., octadecyltrimethylammonium, hexadecyltrimethylammonium, hyamine, and 1-hexadecylpyridinium) were also tested as ion-pairing cations with naproxenate for use as possible liquid ion exchangers in the construction of the electrode. The tetraheptylammonium-naproxenate ion-pair complex was found to have the optimum characteristics with respect to slope, detection limit, and selectivity. The electrode exhibited a rapid and near-Nernstian response to naproxenate activity from 10^{-1} to 10^{-4} M at pH 9.0 (borate buffer). No significant interference from common ions and tablet excipients was found and the electrode could be used for the direct assay of naproxen tablets by the calibration graph method and of suppositories using the standard addition technique. The precision (relative standard deviation) of the potentiometric method was 0.8 and 2.9% ($n = 3$) for tablet and suppository assays, respectively.

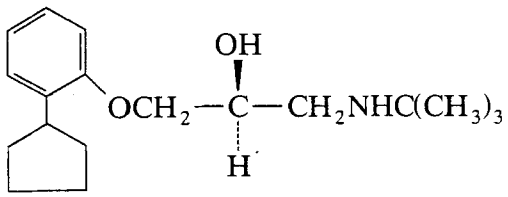
The simplicity, rapidity, and reliability of the proposed potentiometric method were evaluated by means of dissolution studies of naproxen tablets.[94] The results obtained with the proposed potentiometric method for three dissolution experiments on tablets from the same batch compared favorably with those given by the spectrophotometric USP method. From the results obtained, the dissolution rate constant, k_d, could be calculated using the equation

$$C_t = C_f(1 - e^{-k_d t}) \qquad (21)$$

where C_t is the corrected drug concentration of dissolved naproxen at various time intervals (the concentration of naproxen was corrected for the volume of solution withdrawn from the dissolution vessel) and C_f is the final theoretical concentration expected. Analysis of the obtained data gave values for k_d of 0.0261 ± 0.0021 min^{-1} ($r = 0.98$) for the potentiometric method and 0.0267 ± 0.0021 min^{-1} ($r = 0.98$) for spectrophotometric method (pH 7.4, phosphate buffer).

JJ. Penbutolol

$C_{18}H_{29}NO_2$ (MM = 291.4)



Therap. category: antihypertensive, antianginal, antiarrhythmic

Discussion and Comments

The construction as well as the electroanalytical performances of a penbutolol-selective membrane electrode containing

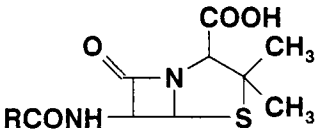
penbutolol dinonylnaphthalene sulfonate ion-pair complex incorporated into a PVC matrix was recently described by Ionescu et al.[94a] The electrode exhibited a Nernstian response in the range of 10^{-3} to 5×10^{-6} *M* penbutolol with a detection limit of 10^{-6} *M*. Its good selectivity toward many inorganic and organic ions and common excipients used in pharmaceutical practice makes it applicable for penbutolol assay from bulk drug substance as well as from pharmaceutical formulations such as tablets. Additionally, the penbutolol membrane electrode proved useful for the determination of dissolution profiles of solid formulations containing penbutolol sulfate. In order to find out the dissolution mechanism of penbutolol coated tablets, the data were numerically solved by using typical simulated equations.

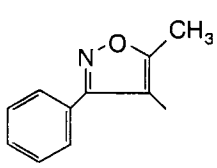
KK. Penicillins

It is well known that antibiotic penicillins (see Table 12) are derivatives of thiazole which may hydrolyze easily, especially in an acidic medium or under the influence of penicillinase. The hydrolysis results in opening of the β-lactam ring that is characteristic of these compounds (see also the previous reviews[8,10] and monograph[11]). Some new papers describe the construction and application of various membrane sensors sensitive to penicillins, most of them based on the penicillin hydrolysis reaction with a result of penicilloic acid formation which is sensed by a primary pH electrode.[95-102]

Yao et al.[95] reported ion-selective electrodes sensitive to penicillins and based on the use of quaternary ammonium, phosphonium, or arsonium ions as the exchange site. Cetyltrioctylammonium (CTOA), cetyltrioctylphosphonium (CTOP), and cetyltrioctylarsonium (CTOAs) were evaluated in order to compare their response characteristics. Penicillin electrodes based on CTOA were found to give the best response with respect to linear concentration ranges, slopes, and detection limits. The electrodes were successfully applied for the determination of penicillin drug content in injectable solutions. The results were in agreement with those obtained by the pharmacopial method.

TABLE 12
Some Penicillins Assayed by Membrane Sensors



Compound	R	Formula (MM)
Ampicillin (sodium)	$C_6H_5-\underset{\displaystyle NH_2}{\underset{\vert}{CH}}-NH_2$	$C_{16}H_{18}N_3NaO_4S$ (371.4)
Benzylpenicillin or penicillin G (Na or K)	$C_6H_5-CH_2-$	$C_{16}H_{17}N_2NaO_4S$ (356.4) $C_{16}H_{17}KN_2O_4S$ (372.5)
Oxacillin (sodium, monohydrate)		$C_{19}H_{18}N_3NaO_5S$ (441.4)
Penicillin V (potassium)	$C_6H_5-O-CH_2-$	$C_{16}H_{17}KN_2O_5S$ (388.5)

It was shown in the previous review[10] and monograph[11] that carboxylated PVC (PVC-COOH) containing 1.8% —COOH groups could be used as a polymer matrix for a pH-sensitive coated-wire electrode and that, using the electrode, a penicillin sensor could be prepared by immobilizing penicillinase on the polymer surface mainly through electrostatic attraction. The carboxyl group in PVC-COOH is essentially required to bind penicillinase on the membrane surface without inactivation. Change of H^+ concentration due to enzymatic hydrolysis of penicillin was detected as a local pH change by the pH-sensitive coated-wire electrode. Accordingly, the pH and the concentration of the working buffer should be crucially important factors affecting the performance characteristics of the sensor. Chen et al.[97] studied the effects of buffer concentration and pH on the potentiometric response of the penicillin sensor based on the PVC-COOH/TDDA-coated Ag electrode. The sensor was prepared by coating the top of the Ag wire, mounted in a Teflon rod, with solvent polymeric membrane composed of PVC-COOH (31%), *o*-NPOE (63%), TDDA (5.5%), and NaTPB (0.5%) and then with penicillinase solution. (The top of the electrode was immersed in 0.5% penicillinase solution in 1 m*M* phosphate buffer, pH 7.0, for about 15 h at 5 to 10°C).

Electrode response to penicillin concentration was highly affected by the buffer concentration. Higher concentration buffer (30 m*M*) strongly disturbed the response due to the higher buffer capacity which cancels the pH change originating from the enzymatic reaction at the electrode surface. It was recommended to use buffer solutions with a rather weak buffer capacity.[97]

In a recent interesting study, Taguchi et al.[98] investigated different immobilization methods in order to construct a penicillin biosensor based on a TDDA/PVC-COOH pH coated-wire electrode (CWE). The methods investigated were:

1. Adsorptive immobilization of penicillinase on pH-CWE. The pH-CWE was dipped overnight at 4°C in 0.3% penicillinase solution in 1 m*M* potassium phosphate buffer, pH 6.7).
2. Covalent immobilization of penicillinase on pH-CWE. The pH-CWE was dipped overnight at room temperature in 4 mg/ml penicillinase solution in water containing 4 mg/ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, EDC.
3. Covalent immobilization of penicillinase on BSA-modified pH-CWE. The pH-CWE was dipped overnight at room temperature in 4 mg/ml BSA solution in water containing 4 mg/ml EDC. The BSA-modified CWE thus obtained was dipped in 5% glutaraldehyde solution (in 5 m*M* phosphate buffer, pH 7.0) for 3 h at 30°C and then dipped in 4 mg/ml penicillinase solution (in 5 m*M* potassium phosphate buffer, pH 7.0) for 1 h at 30°C.

The response time was between 5 and 10 min in each case, irrespective of the immobilization method. The sensor obtained by method c was much better than those obtained by methods a and b. According to the method c, the CWE surface was previously modified with BSA and the main functional groups for immobilization were amino in BSA, which may immobilize penicillinase at a larger extent. In addition, BSA may act as a spacer for the enzyme and steric hindrance may not occur.[98] A stable, large potential change is obtainable with such a sensor even in salt-containing samples (0.15 *M* NaCl in 5 m*M* potassium phosphate buffer, pH 7.0). The possibility of the practical use of this penicillin sensor was suggested.[98]

Penicillin V was determined in a flow-injection manifold by hydrolysis of the β-lactam ring by means of an on-line reactor containing immobilized penicillinase with detection of the produced acid by a glass pH electrode.[96] The penicillin concentration was calculated as a difference in response between a sample passing through the enzyme reactor and a sample flowing directly to the glass electrode. The pH signal was made

linearly dependent on the acid concentration by using a buffer mixture of constant buffer capacity and the reactor was designed so that hydrolysis of penicillin was mainly completed in the reactor. The linear range was 0.1 to 15 mM penicillin and sensitivity was 0.056 pH/mM. The deviation from the linearity above 15 mM is due to an increase of the buffer capacity of the enzyme/glass conjugate below pH 7.

The stability of the immobilized enzyme was estimated by comparing the steady-state kinetic constants before and after daily use for 2 months (> 5000 injections). The maximum apparent rate constant decreased to about half the original value during this time, but the analytical performance was not affected because of the initial overcapacity of the reactor. The activity decreased only a few percent for preparations stored at 4°C, in 0.1 M phosphate buffer, pH 7, during the same time.[96] The proposed method was primarily intended for the analysis of purified potassium salts of penicillins in pharmaceutical preparations.

The construction of a penicillinase electrode with a response time much shorter than those of any previously reported penicillin sensors was recently described by Meier et al.[99] It is based on a glass electrode and is designed to measure penicillin V concentration in phosphate buffer. The glass electrode to be coated with the enzyme penicillinase, after a previous soaking in an enzyme solution containing 1430 units/ml of penicillinase and drying at 4°C, was mounted horizontally in a rotator as shown in Figure 8. An aqueous solution of glutaraldehyde (2.5%) was then sprayed over the sensitive end of the electrode using an air brush, under a pressure of 1.5 bar of nitrogen at room temperature, keeping the electrode in rotation at 50 rpm. The reticulation of the enzyme in the presence of glutaraldehyde over the electrode was allowed to continue for 15 min at 4°C before rinsing the electrode in the buffer (0.01 M phosphate–0.1 M NaCl) at room temperature for 5 min. The electrode was stored in the same buffer at 4°C when not in use.

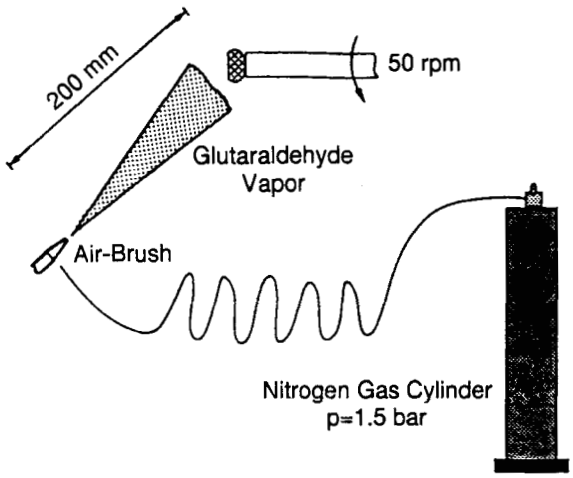


FIGURE 8. Preparation of the penicillinase electrode by spraying a solution of glutaraldehyde over a sensitive end of the electrode previously dipped in enzyme solution. (Reproduced from Meier, H. et al. *Anal. Chim. Acta* **1991**, *249*, 405. With permission from Elsevier Publishing Co.)

The described method of immobilization ensured deposition of a very thin layer (1 to 2 μm) of enzyme on the electrode. Because the response time of the electrode is a function of L^2/D, where L is the thickness of the enzyme layer and D is the diffusion coefficient of the analyte within the enzyme layer, a very short response time (< 10 s) was recorded. The electrode response in phosphate buffer of pH 7.0 was linear in the substrate concentration range from about 1.5 to 15 mM and it showed excellent stability for the first 12 days. A similar electrode was used for the determination of penicillin V in standard solutions and in fermentation broth by flow injection analysis.[100]

LL. Peptides

Peptides are polyamino acids with molecular weights < 5000. They have from two (dipeptide) to 20 to 40 amino acids connected together by amide linkages known as peptide bonds.

A biosensor for peptide determination was recently constructed with trypsin or α-chymotrypsin immobilized on a hydrogen

ion-selective coated-wire electrode and its functional behavior was investigated with two artificial peptides (benzoyl-L-arginine ethyl ester, [BAEE] and *N*-acetyl-L-tyrosine ethyl ester [ATEE]).[103] It is well known that trypsin enzyme hydrolyzes peptides, amides, and esters at bonds involving carboxyl group of L-arginine or L-lysine, and α-chymotrypsin enzyme hydrolyzes them preferentially at the carbonyl end of the hydrophobic amino acids, e.g., L-tyrosine, L-tryptophan, L-phenylalanine, and L-leucine. With artificial substrates, trypsin and α-chymotrypsin catalyze, respectively, the reactions

$$\text{Bz—Arg—OEt} + \text{H}_2\text{O} \rightarrow \text{Bz—Arg—COO}^- + \text{EtOH} + \text{H}^+ \quad (22)$$

$$\text{AcO—Tyr—OEt} + \text{H}_2\text{O} \rightarrow \text{AcO—Tyr—COO}^- + \text{EtOH} + \text{H}^+ \quad (23)$$

The H^+ concentration is increased in both reactions and the concentration of the substrate is directly related to the pH change which is monitored by the hydrogen ion-selective electrode.

Covalent immobilization of the enzyme to the pH coated-wire electrode was made after a previous dipping overnight in bovine serum albumin (4 mg/ml) in order to increase the amount of enzyme immobilized (an amino group of BSA and a —COOH group of PVC-COOH on the coated-wire electrode reacted to form a peptide bond; thus, the number of the reactive groups on the coated-wire electrode for proteolytic enzyme immobilization was increased). Enzyme immobilization required 1 h at 30°C, in 5 m*M* potassium phosphate buffer of pH 7.0.

Response curves of trypsin-immobilized electrode to BAEE and of α-chymotrypsinimmobilized electrode to ATEE, respectively, showed linear ranges of 0.1 to 20 and 0.1 to 40 m*M*, respectively (the potential changes reached constant values within 5 to 10 min in both cases).

The same enzymes were chemically immobilized on the gate surface of a pH ion-selective field effect transistor (*p*-silicon wafer, 0.5 mm wide, 6.5 mm long, and 0.2 mm thick). The ISFET gate was covered with a gel membrane composed of a mixture containing 10% trypsin or α-chymotrypsin, 10% bovine serum albumin in 10 m*M* phosphate buffer of pH 7.0, and 8% glutaraldehyde.[104] BAEE could be determined with the trypsin enzyme electrode in the range $5 \times 10^{-4} \times 10^{-2}$ *M*, while ATEE with the α-chymotrypsin enzyme electrode was determined in the range 5×10^{-4} to 10^{-3} *M*. The response of the sensors was highly affected by pH and the concentration of the working buffer. The experiments showed that the use of a low buffer concentration (e.g., 2 m*M*) is of benefit to determine substrate concentrations lower than 10^{-3} *M*. Unfortunately, the good performances of these devices were limited to about 1 week.

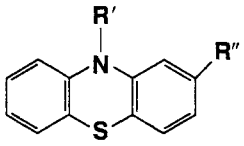
MM. Phenothiazine Derivatives

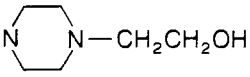
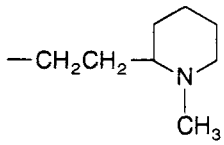
Only a few papers describe the performance characteristics of new phenothiazine drug membrane electrodes.[53,105] Phenothiazine drugs (two of them are presented in Table 13) are compounds with well known neuroleptic activity. Chlorpromazine remains the most widely used antipsychotic drug throughout the world and continues to serve as a standard with which other neuroleptics are compared.

A chlorpromazine membrane electrode constructed by using a modified PVC membrane which has $-SO_3H$ ionic end groups as ion-exchange sites was described by Takisawa et al.[53] (see also details in Section III.T). It responded linearly to chlorpromazine concentration in the range 10^{-3} to 10^{-5} *M* with 57.7 ± 0.2 mV/decade slope (pH range 2 to 6.5). The electrode was used to determine the complexation constant of chlorpromazine hydrochloride with both α- and β-cyclodextrins.

By coating a graphite rod with a film of PVC plasticized with a mixture of dioctylphthalatenitrobenzene and incorporated by

TABLE 13
Some Phenothiazine Drugs Assayed by Membrane Sensors



Phenothiazine, Formula (MM)	R′	R″	Ref.
Chlorpromazine $C_{17}H_{19}ClN_2S$ (318.9)	$-(CH_3)_3N(CH_3)_2$	—Cl	53
Fluphenazine $C_{22}H_{26}F_3N_3OS$ (437.5)	$-CH_2CH_2CH_2-$ 	$-CF_3$	78
Thioridazine $C_{21}H_{26}N_2S_2$ (370.6)		$-SCH_3$	105

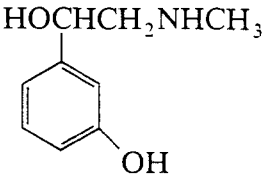
fluphenazine-phosphotungstate ion-pair complex, a fluphenazine membrane electrode was obtained.[78] It responds to fluphenazine cation in the linear concentration range 10^{-3} to 6×10^{-7} M with a 54 mV/decade slope (pH range 3 to 4).

Shoukry and Badawi[105] prepared a thioridazine-coated copper wire electrode based on incorporation of an ion-pair complex of tetraphenylborate anion with thioridazine cation in a PVC matrix. It showed a Nernstian response over a thioridazine concentration range 6.3×10^{-6} to 2.5×10^{-3} M at 25°C and was selective, precise, and usable within the pH range 2.1 to 7.0.

It is well known that aqueous solutions of phenothiazine drugs including thioridazine are photosensitive; the photoreaction occurring in the thioridazine solutions exposed to daylight did not affect the electrode performance. This reaction was investigated kinetically to determine its rate constant and half-life at different temperatures (the photoreaction appears to follow zero-order kinetics; $k = 5.9 \times 10^{-2}$ mol^{-1} ml^{-1} min^{-1} and $t_{0.5} = 11.74$ min at 20°C.

NN. Phenylephrine

$C_9H_{13}NO_2$ (MM = 167.2)



Therap. category: mydriatic, decongestant with α-adrenergic activity without stimulating effects on the central nervous system

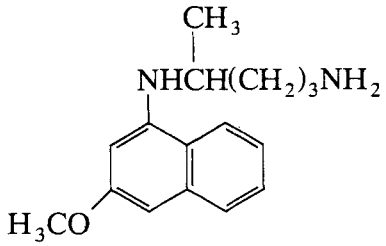
Discussion and Comments

A polymeric membrane electrode selective to phenylephrine was constructed and its performance characteristics were reported.[106] It is based on the incorporation of the lipophilic salt phenylephrine-tetraphenylborate in plasticized PVC matrix (45% DOP as solvent plasticizer; 40% PVC, and 15% ion-pair) and showed near-Nernstian re-

sponse (slope, 57.5 mV/decade) over the concentration range 1.5×10^{-4} to 10^{-1} *M* in solutions of pH 2.9 to 8.0 at 25°C. The proposed electrode was used successfully for the assay of pharmaceutical preparation phenylephrine eye drops (10% solution) by applying the simple extrapolation method on a proper calibration graph. The results obtained for the determination of 0.1 to 6.5 mg/ml showed an average recovery of 100.1% with a mean coefficient of variation of 1.1% ($n = 4$).

OO. Primaquine

$C_{15}H_{21}N_3O$ (MM = 259.3)



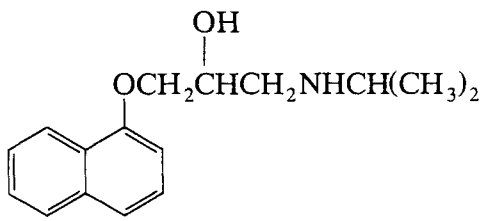
Therap. category: antimalarial

Discussion and Comments

Electrodes based on PVC matrix membranes containing one of three macrocyclic crown ethers with dioctylphenylphosphonate solvent mediator and, in some instances, negative sites have been studied for their potentiometric response to the primaquinium cation.[107] Primaquine ion-selective electrodes based on dibenzo(18-crown-6) gave sub-Nernstian responses, while those based on dibenzo(24-crown-8) (DB24C8) and dibenzo(30-crown-10) (DB30C10) exhibited good electrochemical characteristics such as Nernstian responses, fast dynamic response times (ca. 30 s), a wide range of working pH (pH 4 to 10), and good selectivity over many metal cations, chloroquine, and sulfonamide drugs. The addition of 50% mole ratio negative sites (potassium tetrakis-[4-chlorophenyl]-borate) relative to the crown ether not only led to an improvement of the e.m.f. stability, but also produced, in most instances, improved selectivity characteristics for both the DB24C8- and DB30C10-based electrodes. Hydrophobic sites at less than stoichiometric make full use of the carrier and exclude other anions. Determination of primaquine diphosphate (4.5 to 453.0 μg/ml) using the standard addition method resulted in a mean recovery and relative standard deviation of 107.0 and 8.0%, respectively. Determination of primaquine in pharmaceutical preparations was also described.[107]

PP. Propranolol

$C_{16}H_{21}NO_2$ (MM = 259.3)



Therap. category: β-adrenergic blocking agent, cardiac depressant

Discussion and Comments

The propranolol membrane electrode proposed by Takisawa et al.[53] exhibited a linear concentration range from 10^{-5} to 10^{-2} *M* with 58.8 ± 1.0 mV/decade slope. It was constructed by using a modified poly(vinyl chloride) membrane which has ionic end groups (—SO_3H) as ion-exchange sites and which was cast using a solid polymeric plasticizer (Elvaloy 742, DuPont). Apart from its application in pharmaceutical analysis, this electrode could also be used to investigate the interaction of propranolol with other solute species such as biopolymers and receptors (e.g., α- and β-cyclodextrins).

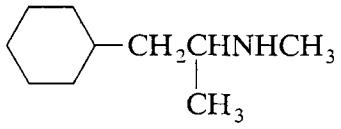
Propranolol was also determined by a kinetic method using a fluoride-selective electrode and based on its reaction with 1-fluoro-2,4-dinitrobenzene at pH 9.0 and 25°C.[33] Initial-slope and fixed-time methods

were used to construct calibration graphs in the range 10^{-4} to 10^{-3} *M* (see also Section III.G). A recovery study performed on synthetic solutions of propranolol with various excipients used in tablet formulations (sugar, sorbitol, galactose, mannitol, lactose, talc, magnesium stearate, etc.) gave a mean recovery of 99.7% (range 98.4 to 102.5%), showing the absence of any interference of these excipients.

Results on the propranolol and similar β-adrenergic blocking agent membrane electrodes[108] prepared with tetra-(*m*-chlorophenyl) borate in the PVC matrix were already discussed in the previous review[10] and monograph.[11]

QQ. Propylhexedrine

$C_{10}H_{21}N$ (MM = 155.3)



Therap. category: adrenergic (vasoconstrictor)

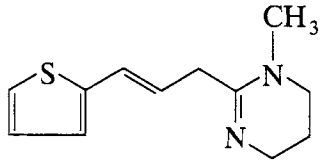
Discussion and Comments

Two types of propylhexedrine membrane electrode were proposed by Zareh et al.[109]: a classical PVC-matrix type, with internal filling solution (10^{-3} *M* propylhexedrine hydrochloride containing 0.1 *M* NaCl) and a Ag-coated wire type. In both cases, the ion-pair complex of propylhexedrine with tetraphenylborate anion in PVC matrix plasticized with DOP was used. Both electrodes showed near-Nernstian responses (54 and 55 mV/decade, respectively) in the range 2.5×10^{-5} to 10^{-1} *M* solutions of the drug (pH 2.5 to 9.5).

Studying the effect of temperature on the electrode performances, Zareh et al.[109] observed that as the temperature raised beyond 60°C, sharp deviation from the Nernst equation was encountered (the slope decreased to 47 mV/decade). This was attributed to collapse of the external thin layer of the membrane which resulted in rapid leaching of the membrane components into the bathing solution. The isothermal temperature coefficient was found to be 0.25 mV/°C.

RR. Pyrantel

$C_{11}H_{14}N_2S$ (MM = 206.3)



Therap. category: anthelmintic

Discussion and Comments

In a recent paper, Aubeck and Hampp[110] reported on the construction, characterization, and comparison of four ion-selective electrodes sensitive to pyrantel. They are based on lipophilic ion-pairs prepared from pyrantel and the inorganic ion-pairing agents reineckate and tungstosilicate and the organic ion-pairing agents tetraphenylborate and dipicrylaminate. A membrane composition of 4.6% electroactive material, 67.1% DBP as plasticizer, and 28.3% PVC was used for electrode preparation. The four electrodes showed similar detection limits of 1 to 2 μg/ml and nearly the same linear ranges of 10^{-5} to 10^{-2} *M* for pyrantel in 0.1 *M* sodium phosphate buffered solutions at pH 7.0. Significant differences between the electrodes were observed in protein-containing solutions. The detection limits of the electrodes with the inorganic ion-pairing agents were not affected by a background of 6.7 g/L (10^{-4} *M*) bovine serum albumin (BSA), but the tetraphenylborate- and dipicrylaminate-based electrodes were 6 to 8 times less sensitive in protein-containing solutions. A high affinity of BSA for dipicrylaminate and tetraphenylborate, and competition at the membrane surface between the primary ion (pyrantel) and BSA were assumed.[110] Alternating measurements of albumin-containing pyrantel samples and albu-

min-free samples with freshly prepared and extensively used electrodes gave no indications of surface contamination of the electrodes by albumin, which could change their electrochemical properties.[110]

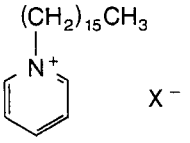
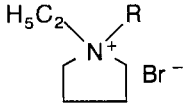
Inorganic cations did not influence the electrode response even when present in high concentrations, but some lipophilic alkaloids, such as berberine and papaverine, did interfere. A pyrantel-silicotungstate based pyrantel membrane electrode was recommended for the determination of pyrantel drug in biological media because it is about twice as sensitive as the pyrantel-reineckate electrode. Those based on organic ion-pair complexes could be used in pharmaceutical process control where the absence of proteins is guaranteed.

SS. Quaternary Ammonium Compounds

A few recent papers[47,111-116] deal with the potentiometric determination of some quaternary ammonium compounds, most of them used as antiseptics and disinfectants in pharmaceutical practice (see Table 14). Cetyltrimethylammonium (cetrimonium) bromide was determined by a kinetic-potentiometric method with a fluoride-selective membrane electrode on the basis of its catalytic effect on the slow reaction of phenylalanine with 1-fluoro-2,4-dinitrobenzene (FDNB)[47] (see also Section III.E). Either initial-slope or fixed-time measurements were used to determine cetrimonium bromide in pharmaceutical formulations such as solutions, tinctures, and ointments. The results obtained were in good agreement with those of the official USP methods and the precision was 1 to 2% ($n = 3$).

Badawi et al.[115] developed and tested a cetrimonium cation-sensitive electrode based on a plasticized PVC membrane containing the dissolved ion-association complex of cetrimonium with phosphotungstate (DOP as solvent plasticizer). The electrode based on 15% ion-pair complex, 42.5% DOP, and

TABLE 14
Some Quaternary Ammonium Compounds Assayed by Membrane Sensors

Compound	Formula (MM)	Ref.
Cetrimonium bromide	$C_{19}H_{42}BrN$ (364.5) $[CH_3(CH_2)_{15}N^+(CH_3)_3]Br^-$	47, 115
Cetylpyridinium chloride (bromide)	$C_{21}H_{38}ClN$ (340.0) $C_{21}H_{38}BrN$ (384.5) 	113, 114
N-Alkyl-*N*-ethyl-pyrrolidinium bromides	 R = C_6H_{11} (hexyl) C_8H_{15} (octyl) C_9H_{17} (nonyl) $C_{10}H_{19}$ (decyl) $C_{11}H_{21}$ (undecyl) $C_{12}H_{23}$ (dodecyl) $C_{15}H_{29}$ (pentadecyl) $C_{16}H_{31}$ (hexadecyl) $C_{18}H_{35}$ (octadecyl)	116

42.5% PVC exhibited a rapid and near-Nernstian response to cetrimonium from 3.2×10^{-6} to 8.3×10^{-4} M in the pH range 2.5 to 8.5 and it has been successfully applied for the determination of the surfactant in aqueous solutions either by the standard addition method or by potentiometric titration with 5×10^{-4} M phosphotungstate with standard deviation ranging from 0.5 to 1.2% ($n = 6$).

Cetylpyridinium chloride was potentiometrically titrated with silver nitrate solution in the presence of a silver-selective electrode.[113] It was observed that the potential of the electrode shifted into the negative region with increased concentrations of cationic surfactant in solutions containing KI. This effect is caused by complexation of iodide with surfactant cation to form negative charged species of type $QI_n^{(n-1)-}$ which are absorbed on the electrode surface (Q = cetylpyridinium). This was confirmed by potentiometric titration of the KI-cetylpyridinium chloride system with $AgNO_3$ solution. The titration curve showed steps corresponding to the following processes: formation of a mixed complex of I^--Q^+-Ag^+, formation of AgI with free I^- in the system, and formation of AgI with I^- liberated from the negative charged species, $QI_n^{(n-1)-}$. This was used for the analytical determination of QCl using the first step on the titration curve. The recommended procedure requires 10^{-4} to 10^{-5} M QCl in a volume of 50 to 100 ml, 10^{-2} to 10^{-3} M KI and $AgNO_3$ concentration comparable to the KI concentration.

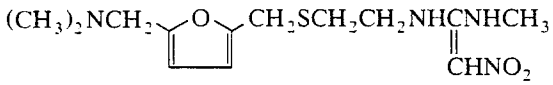
Shoukry[114] titrated QBr potentiometrically with a standard solution of sodium tetraphenylborate ($c = 10^{-2}$ M) in a cell containing two membrane electrodes that were selective for Q^+ and TPB^- ions. Titration curves with sharp peaks were obtained, enabling the equivalence points to be located precisely. The coefficients of variation did not exceed 1.1% and recoveries of 99.6 to 100.1% were obtained ($n = 4$).

Cations of the homologous series of *N*-alkyl-*N*-ethyl pyrrolidinium salts were also determined with a good precision by a titration using the same titrant as above, but with $c = 5 \times 10^{-2}$ M and by using a simple PVC membrane coated-wire electrode as a potentiometric indicator sensor.[116] The titration curves were well defined in all cases; their shapes remained almost the same, but the value of the end-point potential breaks increased significantly with increasing number of carbon atoms of the alkyl chain. In titrations of the homologous series listed in Table 14, the increase of the potential break was proportional to the number of carbon atoms of the alkyl chain up to $n_C = 15$. The assay values were closed to 99% with relative standard deviations not higher than + 1.0% ($n = 5$).

To explain the role of the equilibria involved in the potentiometric titration, the values of both distribution ratios and the extraction constants as well as the solubility products of *N*-alkyl-*N*-ethyl-pyrrolidinium tetraphenylborates were determined by Vytras et al.[116] The value of the titration break could readily be correlated with the logarithmic values of the extraction parameters.

TT. Ranitidine

$C_{13}H_{22}N_4O_3S$ (MM = 314.4)



Therap. category: antagonist (to histamine H_2 receptors) especially in treatment of duodenal ulcer

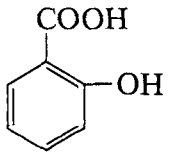
Discussion and Comments

A ranitidine membrane electrode with ranitidine-tetraphenylborate ion-pair complex in PVC matrix displayed a linear response covering the concentration range from 3×10^{-5} to 3×10^{-2} M in KH_2PO_4-borate buffer of pH 9.0.[117] The precipitate constitution of the electroactive material was investigated by mass spectrometry and the surface structure of the membrane electrode was studied by electronic microscopy. Many "pumps" formed on the surface of the PVC membrane electrode were regarded as elec-

troactive centers. The lifetime and reproducibility of the electrode have been discussed in detail.

UU. Salicylic Acid

$C_7H_6O_3$ (MM = 138.1)



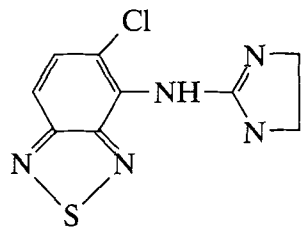
Therap. category: topical keratolytic

Discussion and Comments

Several PVC-based salicylate ion-selective electrodes were constructed and characterized by Petho.[118] Various ion exchangers used for membrane preparation (e.g., tetraoctylammonium-, cetyltrimethylammonium-, tetrabutylammonium-, cetylpyridinium-, physostigminium-, triphenyl-ethylphosphonium-, and benzalkonium-salicylate) were prepared by an extraction procedure and used in a percentage of 5% with different common plasticizers (70%). The linear response range was obtained with tetraoctylammonium ion-pairing agent and *o*-NPOE as plasticizer (10^{-1} to 10^{-4} *M*).

VV. Tizanidine

$C_9H_8ClN_5S$ (MM = 253.7)



Therap. category: muscle relaxant (skeletal)

Discussion and Comments

Three types of polymeric electrodes were constructed using a PVC film plasticized with a dioctylphthalate-nitrobenzene mixture and containing an ion pair (e.g., phosphotungstate-, silicotungstate-, or tetraphenylborate) of the drug-tizanidine.[119] The membrane was prepared by dissolving PVC in cyclohexanone (0.32 g in 4 ml). A 1-ml volume of DOP, 1-ml nitrobenzene, and 20 mg of the ion pair were successively added; this last amount corresponds to 1×10^{-3} *M* concentration of the active material in the membrane. The membrane of the so-called conventional electrode, which includes an internal Ag/AgCl reference electrode, was immobilized on a micropipette tip. A single-layer electrode was prepared by coating a 2-mm-diameter pyrolitic graphite rod with the membrane. The graphite rod of the two-layer electrode was previously coated with poly(4,4′-biphenol) film before the sensitive PVC membrane was obtained by electropolymerization for 1 min by anodic oxidation at 0.95 V vs. SCE from a 5×10^{-3} *M* 4,4′-biphenol monomer solution in acetonitrile containing 0.1 *M* sodium perchlorate. The immersion time in the polymer solution was short because poly(4,4′-biphenol) is slowly dissolved by cyclohexanone. These electrodes exhibited a Nernstian response to tizanidine in the concentration range 5×10^{-6} to 1×10^{-2} *M* with a slope between 55 and 57 mV/decade. The response is not affected by pH change in the range 3 to 7. The influence of several parameters involved in the construction of these electrodes on their lifetime was discussed in detail. The highest durability was observed for the conventional electrode (4 months) and the lowest for the single-layer electrode (1 month). The limited lifetime of the internal solid contact electrode was attributed to a loss of plasticizers; the thin film of the single- and two-layer electrodes was altered more rapidly than the membrane of the conventional electrode, which is protected by the micropipette tip.[119] The lifetime of the two-layer electrode, which is better than that of the single-layer electrode, is due to the stronger adhesion of the sensing membrane,

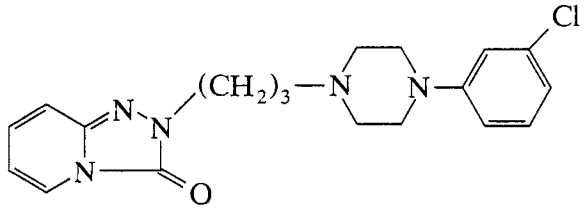
which is fixed in the former instance on another film, the poly(4,4′-biphenol).

Some other drugs, such as mexiletine, promethazine, and trazadone, which are not structurally related to tizanidine, showed severe interference in the electrode response, but these drug compounds are never associated with tizanidine in pharmaceutical formulations and do not influence the analytical results.[119] Several compounds exhibiting similar pharmacological properties were also tested, but they did not affect the electrode response; even dantrolene, which includes an imidazoline moiety in its molecule, had no effect.

Direct potentiometry was applied with good results (recovery, 102.8%; rsd = 1.2%, $n = 5$) to the determination of tizanidine hydrochloride in tablets without any pretreatment or extraction steps.

WW. Trazadone

$C_{19}H_{22}ClN_5O$ (MM = 371.9)



Therap. category: antidepressant

Discussion and Comments

A simple assay procedure for the quantitation of trazadone hydrochloride in tablets, without prior separation, has been developed using a trazadone-selective electrode.[120] The electrode is based on trazadone-tetrakis(*p*-chlorophenyl)borate ion-pair complex, DOP, and PVC matrix that were mounted on a PTFE membrane (composition: 65% DOP, 30% PVC, and 5% ion-pair complex). As ion-pairing agents for the trazadone-selective electrode, TPB, TpClPB, TpFPB, picrolonic acid, methyl orange, reineckate, hexanitrodiphenylamine, eosin, and erythrosine were also tested. Among these electrodes, TPB-, TpClPB-, and TpFPB-based electrodes showed good responses, but the last two displayed a lower limit of linear range than the TPB, probably because these ion-pairing agents form more hydrophobic ion pairs with trazadone than TPB (the electrode with trazadone-TpClPB ion pair had a slope of 58.25 mV/decade in the linear range, a detection limit of 5×10^{-6} *M*, and pH range 2.5 to 5.0). The selectivity coefficients determined by the mixed solution method showed that the trazadone-selective electrode exhibited negligible interference from the organic and inorganic cations commonly present in pharmaceutical preparations. Some diluents and excipients normally used in pharmaceutical preparations did not show any interference, as well. The selectivity sequence of the electrode toward alkylammonium cations was consistent with the order of the extractibility of the ion; the selectivity coefficients increased with the number of carbon atoms in the respective cation.

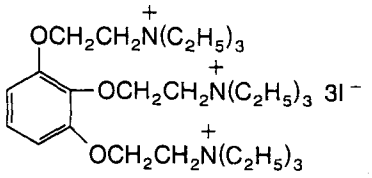
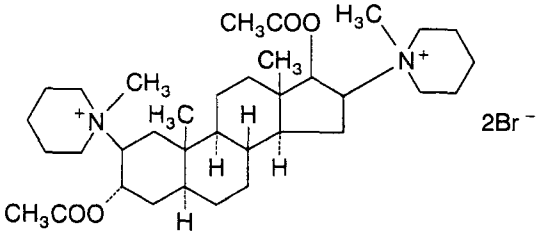
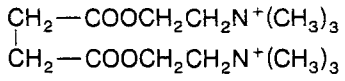
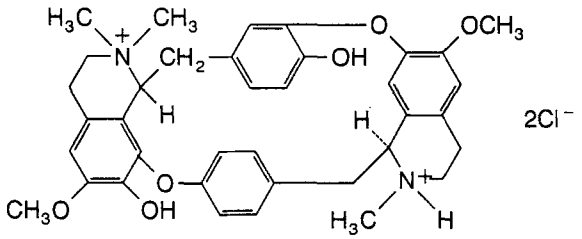
Reproducibility and accuracy were determined by the direct potentiometric method for trazadone solutions in the range 10 to 3000 μg/ml with respect to a calibration graph. The results obtained showed an average recovery of 100.6% and a mean standard deviation of 0.4%. When applied for the determination of trazadone hydrochloride in tablets, the ion-selective method gave comparable results with HPLC method (rsd < 1.0%). The former method offers the advantages of elimination of prior separation steps and low cost of operation compared with HPLC.[120]

XX. Tubocurarine and Related Drugs

Ion-selective PVC membrane electrodes for the peripheral muscle relaxants listed in Table 15 were recently proposed by Aubeck et al.[121]

Ion-pairs of the muscle relaxants with tetraphenylborate and dipicrylaminate as anionic counterions were used as electroactive materials in the membrane (DBP as plasticizer). The detection limits for all electrodes

TABLE 15
Tubocurarine and Related Drugs Assayed by Membrane Sensors

Compound	Formula	MM
Gallamine triethiodide	$C_{30}H_{60}I_3N_3O_3$ 	891.6
Pancuronium bromide	$C_{35}H_{60}Br_2N_2O_4$ 	732.7
Succinylcholine bromide	$C_{14}H_{30}Br_2N_2O_4$ 	450.2
Tubocurarine chloride	$C_{37}H_{42}Cl_2N_2O_6$ 	681.7

were ca. 10^{-6} M at physiological pH values. The observed slopes were the same, regardless of whether tetraphenylborate or dipicrylaminate was used as counterion and were close to the values theoretically expected. Changes in the detection limits and the slopes of the electrodes in the pH range 2.5 to 11.0 were found to be due to reversible changes in the protonation state of the amine groups of the tubocurarine drug or the dipicrylaminate counterion. The pancuronium-tetraphenylborate and pancuronium-dipicrylaminate ion-pair complexes were observed to have a better stability than those formed with tubocurarine, gallamine, and succinylcholine. The observed selectivity coefficients of the pancuronium-tetraphenylborate electrodes were $10^{-0.3}$ toward tubocurarine and $10^{-1.8}$ toward gallamine.

A potential application of such electrodes *in vivo* might be drug monitoring (e.g., with microelectrodes) in pharmacological and anesthetic studies.[121]

YY. Valproic Acid

$C_8H_{16}O_2$ (MM = 144.2)

$(CH_3CH_2CH_2)_2CH—COOH$

Therap. category: anticonvulsant; antiepileptic (as Na or Ca salt)

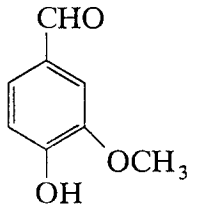
Discussion and Comments

A simple and rapid method for the determination of sodium valproate in pharmaceutical preparations, without prior separation has been developed using a valproate-selective electrode.[122] A plastic liquid membrane electrode kit was used for the electrode based on the use of valproate-methyltris (tetradecyl) ammonium ion-pair complex in *n*-decanol and PVC matrix [membrane composition: 67% (w/w) 0.2 *M* ion-pair complex solution and 33% (w/w) PVC]. The electrode showed a near-Nernstian response in the range 10^{-2} to 2×10^{-4} *M* sodium valproate (pH range 6.5 to 11.5) without interference from pharmaceutical excipients and diluents commonly used in drug formulations.

Determination of 90 to 1500 μg/ml of sodium valproate in aqueous solution by direct potentiometry showed an average recovery of 100.0% (mean standard deviation, 0.4%). Commercially available preparations containing sodium valproate were determined by the ion-selective method and compared with the GC method. The results obtained by both methods showed good agreement and precision (rsd ranged from 0.5 to 1.2%, $n = 6$, for ISE method).

ZZ. Vanillin

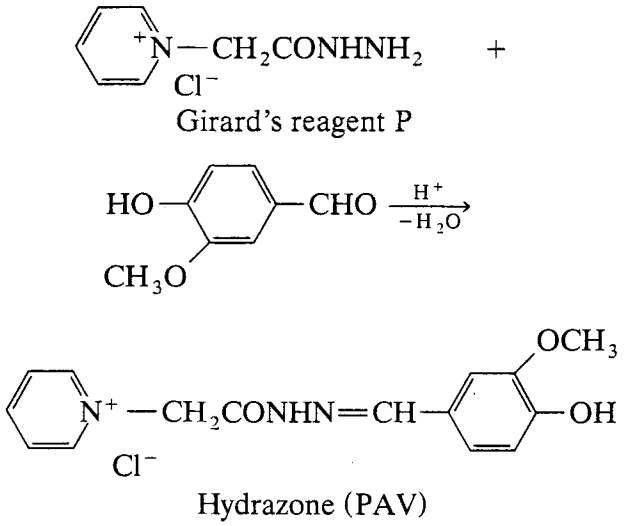
$C_8H_8O_3$ (MM = 152.1)



Therap. category: pharmaceutic aid (flavor)

Discussion and Comments

A hydrazone-selective electrode based on the vanillin hydrazone-tetraphenylborate ion-pair in a PVC membrane (DOS as plasticizer) was proposed for the determination of vanillin.[123] Vanillin was converted to its pyridinium acetohydrazone derivative (PAV) by reaction with Girard's reagent P in the presence of an acid catalyst:



(24)

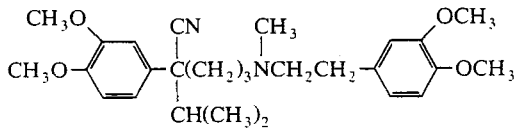
The resulting ionic species was not isolated, but was detected with the PVC membrane hydrazone-selective electrode, thus providing an improved ISE method for the determination of vanillin than that reported earlier[124] (see also the previous review[10] and monograph[11]). Vanillin was first reacted with Girard's reagent P(1-[carbazoylmethyl] pyridinium chloride) to give the corresponding hydrazone in aqueous solution, in the presence of a few drops of glacial acetic acid (reaction time, 30 min at room temperature). With appropriate dilution, the amount of PAV was measured using the electrode method in which the electrode was first calibrated using the same excess of Girard's reagent P for hydrazone generation. The electrode exhibited a sub-Nernstian response (slope, 42.4 mV/decade of vanillin concentration) in the range 1×10^{-2} to 4×10^{-5} *M* vanillin and in presence of twofold excess of Girard's reagent P to allow for complete formation of the hydrazone (the reagent does not interfere in the electrode response even when a fivefold excess is used for hydrazone formation). The protonation of hydrazone

(PAV) depends on the pH of the aqueous medium, which may in turn affect the electrode response toward the positively charged hydrazone. Within the pH range 4.5 to 6.0, the electrode potential varied by < 3 mV and, as a consequence, the electrode was used within this pH range.[124]

The analysis results obtained by the electrode method using two-, four-, and fivefold excess of Girard's reagent P for the *in situ* generation of hydrazone were in good agreement with those given by the UV–AOAC method.

AAA. Verapamil

$C_{27}H_{38}N_2O_4$ (MM = 454.6)



Therap. category: coronary vasodilator

Discussion and Comments

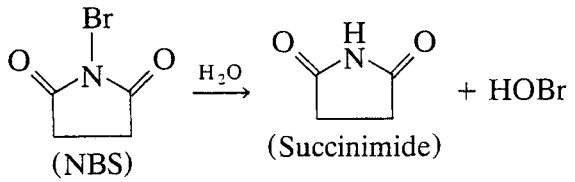
Verapamil membrane electrodes based on the use of verapamil-reineckate[125] and verapamil-tetraphenylborate[126] ion pairs, respectively, were described. With the verapamil-reineckate ion pair, a linear response covering the concentrations range from 10^{-5} to 10^{-2} M, was obtained (sub-Nernstian slope of 52.8 mV/decade). The reproducibility of repeated measurements on the same solution was $\pm$ 0.1 mV. pH has a negligible effect within the range 3.0 to 7.0; at pH > 7.0, verapamil free-base-precipitates and, consequently, the concentration of the protonated species decreases. The electrode was found to be useful in the potentiometric determination of verapamil in pure bulk drug solutions by direct potentiometry using the standard addition technique and potentiometric titration with NaTPB standard solution (average recovery, 99.3%; relative standard deviation, 0.4%).

Using verapamil-tetraphenylborate ion-pair complex as the active material, four other types of verapamil-selective electrodes were prepared: conventional PVC membrane, Pt-wire coated PVC membrane, carbon-rod coated PVC membrane, and Pt-wire coated bilayer polymeric film.[126] The platinum wire was modified by conductive polyaniline and then coated by PVC membrane containing the electroactive material. This kind of electrode showed a Nernstian range from 10^{-2} to 5.7×10^{-6} M with a slope of 58 mV/decade at pH 3.0 to 6.5 (detection limit, 2.5×10^{-6} M).

All the reported verapamil electrodes presented satisfactory selectivity, fast response, and good stability.

BBB. Vitamins

Again, a substantial quantity of research has been done for the determination of vitamins by potentiometric techniques with various membrane electrodes. Table 16 refers to the latest references in this field. A kinetic-potentiometric method applicable for the determination of all vitamins in Table 16 was proposed by Halvatzis and Timotheou-Potamia.[127] The vitamin reacts with *N*-bromo-succinimide (NBS; a brominating and oxidizing agent) and the rate of production of bromide is monitored with a bromide-selective membrane electrode. The change in electrode potential within a fixed period of time is measured and related to the vitamin concentration. (NBS may be regarded as a source of hypobromous acid, which is generated by hydrolysis:

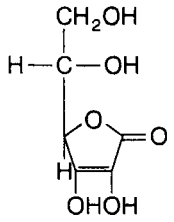
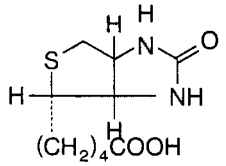
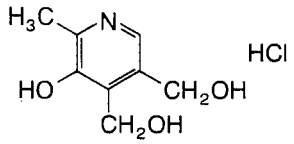
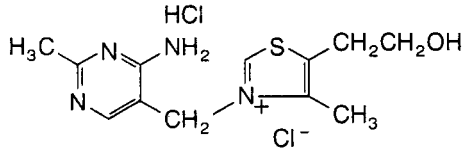


(NBS) (Succinimide)

(25)

HOBr is probably responsible for the oxidizing properties of NBS [the oxidation reactions with NBS are stoichiometric and quan-

TABLE 16
Some Vitamins Assayed by Membrane Sensors

Vitamin	Formula (MM)	Ref.
Ascorbic acid (vitamin C)	$C_6H_8O_6$ (176.1) 	127
Biotin (vitamin H)	$C_{10}H_{16}N_2O_3S$ (244.3) 	127, 132
Pyridoxine hydrochloride (vitamin B_6)	$C_8H_{12}ClNO_3$ (205.6) 	127, 129
Thiamine hydrochloride (vitamin B_1)	$C_{12}H_{18}Cl_2N_4OS$ (337.3) 	127–131

titative and were already used in chemical analysis].)

All experimental parameters affecting the reaction rate of NBS with vitamins (concentration of reagents, reaction time, pH, etc.) were carefully studied and selected.[127] A concentration of 1.5×10^{-3} M NBS was found to be adequate in all cases. For ascorbic acid and biotin, the reaction medium was fixed at pH 3 (0.05 M acetate buffer), while for vitamin B_6 and vitamin B_1 the reaction medium was fixed at pH 8.0 and 7.0, respectively (0.05 M phosphate). The potential change (ΔE) within a fixed period of time (5 s for ascorbic acid and biotin, 25 s for vitamin B_6, and 150 s for vitamin B_1) was estimated graphically by plotting $(10^{\Delta E/S} - 1)$ vs. vitamin concentration (micrograms per milliliter; S is the slope of the electrode response, checked daily with buffered KBr solutions).

Figure 9 shows typical reaction curves for the reactions of NBS with thiamine hydrochloride and ascorbic acid. Biotin and pyridoxine hydrochloride gave similar changes in cell potential vs. time. Results for the determination of vitamins in aqueous solutions showed average errors which did not exceed 2.3% (sd < 2.5% for concentration of 5 μg/ml).

The effect of some common coexisting vitamins on the recovery of 5 μg/ml of vita-

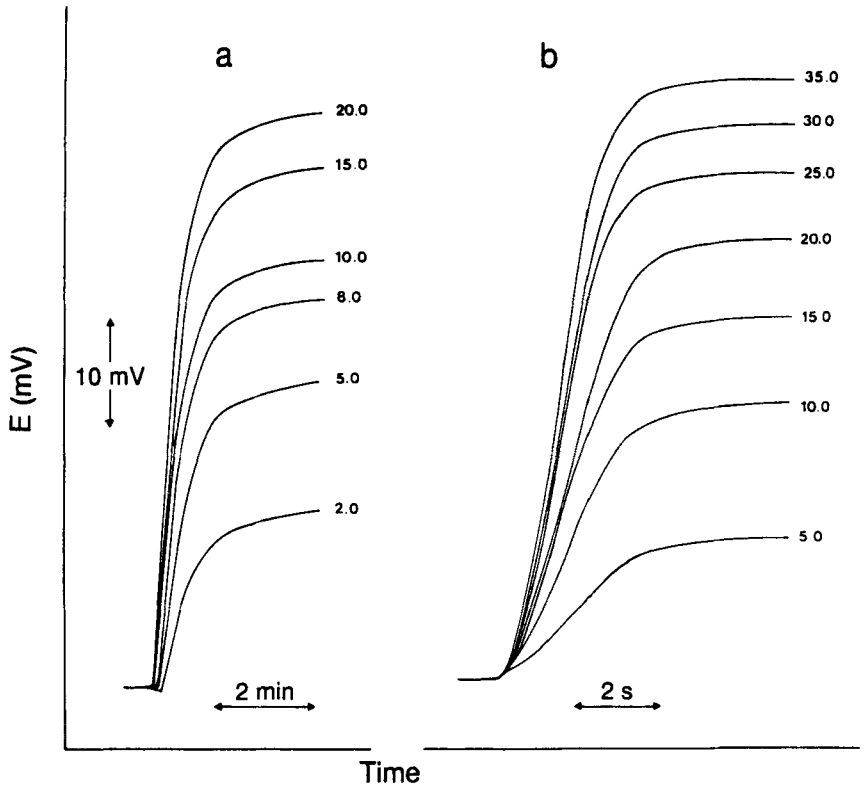


FIGURE 9. Typical reaction curves for the reaction of NBS with (a) thiamine hydrochloride and (b) ascorbic acid at the concentrations (micrograms per liter) shown on the lines. (Reproduced from Halvatzis, S.A.; Timotheou-Potamia, M. *Anal. Chim. Acta* **1989,** *227,* 405. With permission from Elsevier Publishing Co.)

mins listed in Table 16 was studying by analyzing synthetic sample solutions as with excipients, but with various amounts of each coexisting vitamin.[127] Riboflavin and calcium panthotenate do not interfere with the determination of ascorbic acid, biotin, and vitamin B_6, even when present in 20-fold excess, or with the determination of vitamin B_1 when present at a concentration ratio < 10. Nicotinamide interferes only with the determination of vitamin B_6 and if present at a concentration ratio > 1. Vitamin B_{12} interferes with the determination of all the vitamins listed in Table 16 if present at a concentration ratio > 0.1, owing to its reaction with NBS.

The proposed method was evaluated by analyzing commercial formulations of the examined vitamins and the obtained results were compared with those obtained by official methods (USP or BP).

Hassan and Elnemma[128] described a simple and selective argentometric method for the determination of vitamin B_1, based on direct potentiometric titration in alkaline media (> 0.5 *M* NaOH) in which chemical transformation takes place, creating two acidic groups, the protons of which are replaceable by silver ions. Potentiometric titration, performed with 10^{-2} *M* $AgNO_3$ in the presence of a Ag/Ag_2S membrane electrode, gives potentiometric curves with two sharp consecutive potential breaks (ca. 300 and 120 mV, respectively) (Figure 10). The first and second breaks correspond to the consumption of 0.8 ± 0.02 and 1.96 ± 0.02 mol of silver nitrate per mole of vitamin B_1, respectively. Addition of sodium chloride to the thiamine hydrochloride solution before titration does not influence either the nature or the shape of the titration curves, revealing that the curve is not due to the reaction of either of the two dissociable chloride ions in the thiamine hydrochloride molecule.

No interference is caused by other vitamins, active ingredients, or inactive ex-

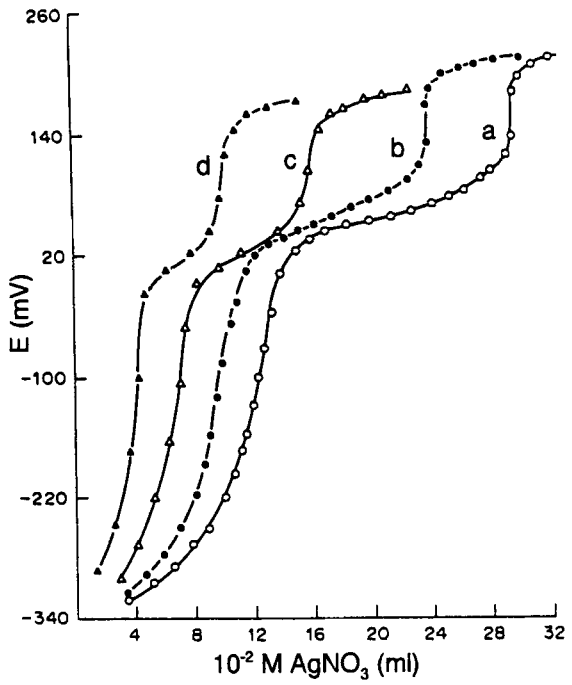


FIGURE 10. Typical potentiometric curves for titration of (a) 15, (b) 12, (c) 8, and (d) 5 ml of 10^{-2} *M* thiamine hydrochloride solution in 0.5 *M* NaOH with 10^{-2} *M* $AgNO_3$ with an Ag_2S membrane electrode. (Reproduced from Hassan, S.S.M.; Elnemma, E. *Talanta* **1989,** *36*, 1011. With permission from Pergamon Press.)

cipients normally present in multivitamin preparations. The results obtained for the determination of thiamine in pure powders, pharmaceutical tablets, and ampoules showed an average recovery of 98.2% of the nominal values, a mean standard deviation of 0.5%, and agreed satisfactorily with data obtained by the BP procedure.

Both dinonylnaphthalene sulfonate and tetra(*m*-chlorophenyl) borate were used as mobile anionic sites to prepare PVC-type membrane electrodes sensitive to vitamin B_1 as well as vitamin B_6 with good performances in respect to linear concentration range and selectivity.[129] Many other tetraphenylborate derivatives were investigated in detail by Zhang et al.[130] in order to construct a vitamin B_1-sensitive PVC membrane electrode. The best electrode was obtained by using as sensing material an ion-pair of vitamin B_1-tetrakis (3,5-bis[2-methoxy-hexafluoro-2-propyl]-phenyl) borate (HFPB) or vitamin B_1-tetra-kis(3,5-bis[trifluoromethyl]-phenyl) borate (TFPB) as a novel electroactive component which was plasticized with *o*-NPOE. The electrodes based on HFPB and TFPB exhibited Nernstian response down to $10^{-7.5}$ and 10^{-7} *M* vitamin B_1 at pH 4, respectively. The lifetime of the electrode based on TFPB, examined by measuring the electrode potential every 10 days, was of at least 490 days. The very long lifetime of this electrode may be due to the high lipophilicity and acid resistivity of TFPB.

The usefulness of the vitamin B_1 electrode was examined by applying the electrode to the determination of vitamin B_1 in an oral solution. The content of vitamin B_1 in this formulation was determined with the electrode in both batch and flow injection systems and was also determined by the conventional HPLC method. Excellent correlations were observed between the results obtained by these methods.[130]

Ion-selective electrodes without an inner reference solution and tubular potentiometric detectors for the determination of vitamins B_1 and B_6 in pharmaceutical preparations by flow injection analysis (FIA) were reported by Lima et al.[131] The membranes were prepared with the vitamin-tetra (2-chlorophenyl) borate (TCPB) dissolved in *o*-NPOE and immobilized on PVC. Intrinsic behavior of the tubular detectors was assessed using a low-dispersion single-channel FIA manifold and was compared with conventionally shaped electrodes using the same membrane. The operating characteristics of these electrodes compared favorably to those of other electrodes reported in the literature (see References 10 and 11) and sensitive either to vitamin B_1 or vitamin B_6.

Data obtained in the determination of vitamin B_1 and vitamin B_6 in pharmaceutical preparations with a double channel flow injection manifold incorporating the tubular detectors demonstrated the feasibility of substituting normally slow and costly reference methods (e.g., USP) with a rapid and easily automated procedure.

A homogeneous competitive binding assay for biotin based on the enzyme adenosine deaminase (ADA) and the strong and specific biotin-avidin interaction was described by

Kjellstrom and Bachas.[132] Unlike conventional homogeneous immunoassays, in this method the analyte (biotin) is labeled with ADA, an ammonia-producing enzyme. Consequently, potentiometric, rather than photometric, methods could be used as means of detection. Several ADA-biotin conjugates were prepared and showed as high as 97% inhibition of the enzymatic activity in the presence of avidin. Addition of free biotin reverses this inhibition in an amount proportional to the concentration of the analyte. Relatively steep dose-response curves were observed, leading to a precise and accurate assay for biotin. The detection limits of these curves were as low as 1×10^{-8} *M*. Varying the concentration of the reagents in the assay allowed the detection limit and working range to be altered to a desired value.[132] The potentiometric method should be advantageous vs. photometric method for the determination of biotin in turbid and colored samples.

IV. CONCLUSIONS

Ion-selective electrode techniques are still showing the feasibility of replacing time-consuming and costly procedures for analysis of drugs and pharmaceutical preparations in complex matrices. In many cases there are definite advantages from using ion-selective electrodes since they can be used for the direct determination of the species in pharmaceutical formulations without the need for complex sample manipulations. The procedure is frequently limited to adjusting the pH and the ionic strength of the solutions in which the determinations are made. Considerable attention has been paid to research in the field of ion-selective polymeric membrane electrodes. An ion-selective electrode containing an ion-pair complex and comprising a plasticized poly(vinyl chloride) membrane is the most suitable type for use as a sensor in the potentiometric determination of many organic ions of biological and pharmaceutical interest. Many drugs are known to stimulate central nervous system activity and they have a significant potential for abuse and tolerance. Their dependence-producing property leads to physiological disintegration. The detection of such drugs in the urine of offenders, which are quantified most commonly by GS-MS after pre-extraction and purification steps, is a time-consuming work requiring special training. The potentiometric methods using selective drug membrane sensors are more attractive for routine analysis. As such, compounds may be determined easily with no associated sample color or turbidity problems and hence, the samples require no pretreatment. This advantage should make a valuable contribution not only in pharmaceutical analysis and research but in forensic chemistry as well. Research on finding new electroactive materials to enable us to achieve very low detection limits ($\leq 10^{-6}$ *M*) has to be carried out. Additionally, the development of a potentiometric ion-selective electrode that can be calibrated to read the enantiomeric purity of a chiral analyte is an attractive target, as well.

Compactness of high performance biosensors sensitive to various drugs is another important target in order to allow them to monitor a certain ionic activity in a continuous bedside way. The utilization of combined multisensor systems in artificial organs, *in vivo* utilization, is of great importance for the near future.

An ion-sensitive field effect transistor (ISFET) could be made sensitive to some organic substrates by immobilizing a suitable enzyme on the gate surface. Because of the high selectivity originating from the highly specific enzymatic reaction, microenzyme drug sensors based on the ISFET have proved to be useful in pharmaceutical and biomedical analysis for a variety of substrates.

The combination of ion-selective electrodes with flow injection or with kinetic (reaction-time) methods of analysis has proved to be a very attractive technique. The selectivity of a membrane electrode combined with, e.g., the selectivity, rapidity, and flexibility of kinetic methods, provides selective analytical methods having increased measurement throughput.

The authors expect that new trends in drug membrane sensors and related instrumentation development will stimulate their use for *in vivo* measurements in order to perform pharmacokinetic studies.

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