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Voltammetry at Liquid / Liquid Interface

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1. Introduction

Recent elecrochemical studies on ion transfer reactions across the interface between two immiscible electrolyte solutions, or, in short, the oil/water(o/w) interface, like nitrobenzene/water and 1,2-dichloroethane/water interface, have shown that the o/w interface can electrochemically be polarized and that the transfer of ions that takes place across the o/w interface within the polarizable potential range, that is, in the span of what is called the potential window can be studied by use of voltammetric or polarographic techniques. In the presence of an ionophore L in O phase, L(O), which selectively associates with a specified ion M in W phase, M(W), to form a hydrophobic complex ML in O phase, ML(O)(and, in some cases, $L_nM(n=2,3\cdots)$ and the like), that is, L(O) $+M(W)=ML(O)$, the o/w interface can be made selectively transferable to the specified ion M. Thus, the o(with ionophore)/w interface can function as an electrode interface which responds voltammetrically to the specified ion (or ions) that is(are) transferable across the interface. In other words we have an ion-selective electrode based on the polarizable o/w interface. Thus, according to the theory of voltammetry, there are available two types of the ion-selective electrode: the amperometric ion-selective electrode and the potentiometric ion-selective electrode. The former gives a current response which is proportional to the concentration of analyte ion, whereas the latter gives a potential response which changes linearly with the logarithm of the concentration(strictly stating, the activity) of analyte ion. [1-3] In this article some recent advances of voltammetry, particularly those of amperometry at liquid/liquid interface, with some emphasis on certain subjects in which our research interest has been concerned, will be discussed. In the first part, electrochemical sensors and biosensors based on amperometric ion-selective electrodes are discussed. In the second part, voltammetric studies of organic compounds of biological interest, like uncouplers and neurotropic drugs, are discussed. In the last part, voltammetric study of phospholipid monolayers formed at the o/w interface and its application to the measurement of hydrolytic activity of phospholipase D at the interface are discussed.

2. Amperometric Ion-Selective Electrode(ISE) Sensors

A. Potassium and Sodium ISE Sensor

The electrochemical cell for amperometric determination of potassium and sodium ion with the ISE sensor is represented by [4]

$$\overset{*}{\mathrm{Ag}\,|\,\mathrm{AgCl}\,|\,\underset{0.1\mathrm{M\ MgSO_4}}{0.02\mathrm{M\ TPenACl}}\,|\,\underset{0.02\mathrm{M\ BB15C5}}{0.1\mathrm{M\ TPenATPB}}\,\|\,\underset{\text{(test soln.)}}{0.01\mathrm{M\ MgCl_2}}\,|\,\mathrm{AgCl}\,|\,\mathrm{Ag}} \quad \text{(A)}$$

(W) (NB) HSM (W)

(amperometric K^+ and Na^+ ion sensor)

In this cell TPenACl, TPenATPB and BB15C5 are

tetrapentylammonium chloride, tetrapentylammonium tetraphenylborate and bis[(benzo-15-crown-5)-4'-ilmethyl]-pimelate (an ionophore), respectively. The polarized nitrobenzene(NB)/water(W) interface is marked by*. In the presence of an ionophore BB15C5 at 0.02M in NB the transfer of potassium and sodium ions across the NB/W interface is observed at $E_{1/2}$=0.198 and 0.381 V, respectively, when these ions are present in W(test solution). The polarized NB/W interface is stabilized by placing a hydrophilic semipermeable membrane (HSM, a dialysis membrane 20 μm in thickness, Visking Co.) at the interface. Since a usable reference electrode for organic phase is not available, it is a common practice to use an Ag/AgCl/Cl^-(W) electrode with, for example, $TPenA^-$(W)/$TPenA^-$(NB) interface, making a reference electrode reversible to $TPenA^-$ ion in NB phase. Thus, the left-hand half-cell, Ag/AgCl/0.02M TPenACl, 0.1M $MgSO_4$(W)/0.1M TPenATPB, 0.02M BB15C5 (NB)// represents the potassium and sodium ISE sensor, which is immersed in a test solution that may contain 0.01M $MgCl_2$ as the supporting electrolyte. The presence of the HSM at the interface gives the sensor physical strength and easiness in handling. It also protects the electrode surface from contamination, for instance, by colloidal particles in the test solution. The(minimum) response time of the sensor is determined by the transient change of the surface concentration of analyte ion on the W-side of the polarizable o/w interface, whichin turn is controlled by diffusion process of analyte ion within the HSM.

In the amperometric ISE the flow of response current across the interface results in the change of the state of electrolyte distribution at and near the electrode interface, which is a disadvantage of amperometric ISE sensors especially when the sensors are used for a long period. This can, however, practically be eliminated by use of the pulse amperometric technique[4,5] : the electrode potential is controlled first at the initial potential E_i at which negligible ion-transfer current flows. After a fixed, relatively long waiting time T, *e.g.* 5 s, the potential is changed abruptly to $E_{app}(=\Delta E+E_i)$ at which an ion-transfer current will flow for a short period τ, *e.g.* 100 ms, in duration. The potential pulse is ended by a return to the initial potential E_i and the electrode is kept at E_i until the next potential pulse is applied after the fixed waiting time and so on. Since the ion transfer reaction at the o/w interface is generally reversible, the electrode interface returns to its original state at the end of the waiting time ; thus highly reproducible current response can be obtained for a long term of measurement. The current is sampled usually at a time near the end of the pulse, and a signal proportional to this sampled value is recorded. Thus, the recorded current *vs.* potential curve is equivalent to a normal-pulse polarogram in polarography. When the applied potential is sufficiently large to give the limiting current, the current signal is directly proportional to the concentration of analyte ion M in test solution (W). The pulse amperometry technique appears essential to obtain reproducible results with amperometric ISE sensors.

When two or more ion components that give the current response at a one and same amperometric ISE are present in test solution, the ISE sensor gives the current responses of the components independently each at their half-wave potentials. Therefore, if their half-wave potentials are reasonably separated, the concentration of these components can simultaneously be determined with a one and same ion sensors. This is actually the case of the present potassium and sodium ion sensor, since $E_{1/2,\,Na}-E_{1/2,K}$=0.183 V. For simultaneous determination of two analytes the dual pulse amperometry technique can be used [4].

With the present laboratory-made potassium and sodium ion sensor the calibration curves, after correction for residual currents, were linear for both ions up to the concentration of 0.7 mM, beyond

which the curves deviated downward from the linearity. The upper limit of the linearity should be improved by elevating the concentration of ionophore in the organic phase. The relative standard deviation was 1.84% (n=5, at 0.3 mM) and the detection limit was $3\sigma=0.02$ mM. The response time was ca. 20s. [4]

Analytical application of the potassium and sodium ion sensor to food chemistry as well as clinical chemistry is interesting. [4,6,7] For practical purpose organic solvents of lower vapor pressure, such as o-nitrophenyloctylether, o-nitrophenylphenylether and 2-fluoro-2'-nitrodiphenylether, can be used. [8] Recently, an amperometric lithium ISE sensor and its application to separate determination of lithium and sodium ions have been studied.[9]

B. Ammonia and Volatile Amine ISE Sensor

The electrochemical cell for amperometric determination of ammonium ion or ammonia gas with this ISE sensor is represented by [10]

$$\mathrm{Ag|AgCl|0.1M\ TBACl}\left|\begin{matrix}\mathrm{0.1M\ TBATPB}\\ \mathrm{0.02M\ DB18C6}\end{matrix}\right\|^{*}\begin{matrix}\mathrm{0.05M\ MgCl_2}\\ \mathrm{0.05M\ L\text{-}Lys}\end{matrix}\left\|\begin{matrix}\mathrm{Test\ Soln}\\ \mathrm{(pH\ 9.0)}\end{matrix}\right. \qquad (B)$$

(W) (NB) HSM ↑ (W) GPM

Ag|AgCl|

(amperometric NH_4^+ ion and NH_3 sensor)

where TBACl and TBATPB are tetrabutylammonium chloride and tetrabutylammonium tetraphenylborate, respectively. The polarizable o/w interface (marked by *) is stabilized by placing a HSM at the o/w interface and the ammonium-ISE surface is covered by a gas permeable membrane (GPM, a Teflon membrane 50μm in thickness, Sumitomo Denko FP-200) with the inner solution of 0.05M $MgCl_2$, 0.05M-L-lysine (pH 8.5) between the GPM and the polarized NB/W(HSM) interface. The counter reference electrode Ag AgCl (W) is connected to the cell through the inner solution. The GPM-covered sensor is immersed in test solution, usually of pH 9.0, into which an aliquot of sample solution is added. The pulse amperometric technique can be used to record the current response of the sensor. The laboratory-made GPM-covered ammonium ion sensor gave the linear current response to the concentration of ammonium ion in test solution. The response time was about 60 s. The large value of the reponse time should be attributable to the diffusion process across GPM and inner solution layer.

The laboratory-made ammonium ion sensor stated above gives a current response also to ammonium ions of other volatile amines like methyl-, dimethl- or trimethylammonium ion. Therefore, the GPM-covered volatile amine sensor can be used to quantify the volatile amine content in foods. Since trimethylammonium ion also gives the ion transfer current at NB(without ionophore)/W interface, separate determination of ammonia and trimethylamine in foods can be made by use of two sensore ; one based on the NB(with ionophore)/W interface and the other on the NB(without ionophore)/W interface. The former gives the sum of the current responses of two amines and the latter that of trimethylamine only. [6,10]

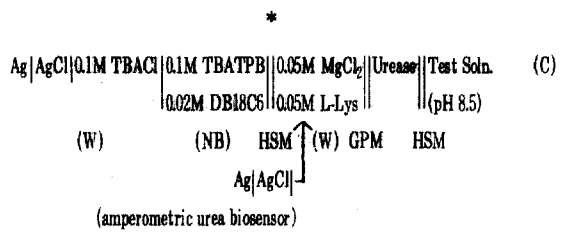
C. Amperometric Ultramicro ISE Sensors

Fabrication of an amperometric ultramicro ISE sensor is feasible by constructing the polarizable o/w interface at the tip, a few tens μm in diameter, of a micro glass pipette.[11, 12] Unfortunately the in vivo voltammetric application of an amperometric ultramicro acetylcholine sensor was unsuccessful mainly because of lack of enough sensitivity. Amperometric micro-hole ISE sensors also appear promising.[11-14]

D. Amperometric Urea Biosensor

A urea biosensor can be constructed by immobilizing urease on the surface of a GPM-covered ammonium ion sensor (cell B,

above). The electrochemical cell for amperometric determination of urea with the urea biosensor immersed in a test solution is represented by.[15, 16]



The electrochemical cell of the GPM-covered ammonium ion sensor is the same as that of the ammonium ion sensor stated above (cell B). Urease (jack bean urease, usually, 100 U) was immobilized by covering the surface of the GPM-covered ammonium ion sensor by a hydrophilic semipermeable membrane (HSM, a dialysis membrane 20 μm in thickness) with a thin, urease solution (0.1M tris-HCl, pH 8.5, 15% bovine serum albumin) layer between the GPM and HSM. The pulse amperometirc technique can be used to record the current response. The biosensor gave the linear current response to urea concentration up to 0.1 mM in the test solution. The relative standard deviation of the current response was 3.8%(n=5, at 20 μM) and the life time was more than 20 days. The urea biosensor was successfully applied to the determination of urea contents in biological fluids. [15]

An advantage of the amperometric urea biosensor is that the correction for the residual current due to the residual ammonium ion that may be present in test solution can relatively easily be achieved compared with the potentiometric ISE sensors. The (normal) urea biosensor, that is, the urease-immobilized urea sensor gives the sum of the current responses each proportional to their concentration when noth urea and ammonium ion are present in test solution. On the other hand, an amperometric (ammonium ion) sensor of the same configuration as the (normal) urea biosensor, but without urease in the immobilized-enzyme layer was fabricated. This urease-removed urea sensor gave a current response proportional to the concentration of ammonium ion present in the test solution but no current response to urea. Thus, the concentration of urea corrected for the residual ammonium ion can be computed from the difference between the current response of the (normal) urea biosensor and that of the urease-removed urea sensor. [16]

A creatinine biosensor can also be fabricated by much the same way as the urease biosensor but using creatinine deiminase in place of urease. [15]

3. Voltammetry of Organic Compounds of Biological Interest

A. Voltammetry of Drugs

A number of organic compounds of biological interest have been studied by use of voltammetry at liquid/liquid interface by many investigators. Thus, voltammetric behavior of procaine at NB/W interface and its analitical application have been studied by Yu and Wang [17] and procaine and its analogues like lidocaine, tetracaine and dibucaine, particularly as a function of pH, by Yamamoto et al. [18] The half-wave potentials of the drugs may reflect the hydrophobicity of these drugs. Takamura and her co-workers have studied the relationship of the drug activity with the half-wave ion-transfer potentials of various drugs at the o/w interface, including local anesthetic, hypnotic, cholinergic, and anti-cholinergic agents. [19, 20]

B. Voltammetric study of uncouplers

Voltammetric behavior of uncouplers of mitochondrial oxidative phospholyration, including 2,4, 6-trinitrophenol(TNP), 2,4-dinitrophenol(DNP), carbonylcyanide p-trifluoromethoxyphenylhydrazine(FCCP), and 3,5-di(tert-butyl)-4-hydroxybenzylidenemalonitril (SF6847), at the o/w (NB/W) interface has been studied, revealing some

physicochemical properties of these compounds relevant to their activity of facilitated transfer of protons at the o/w interface. [21] Also, an equation of the facilitated transport of protons a biological membrane when the uncouplers are added in the medium has been derived, in which the effect of the interfacial potential difference at the two membrane/solution interfaces are explicitly taken into account. The equation has proved to explain the unique behavior of TNP that it is ineffective when added to intact mitochondria but effective when added to submitochondrial particles with inside-out mitochomdrial inner membranes. [22]

4. Voltammetric Study of Phospholipid Monolayers at O/W Interfaces

A. Phase Transition and Ion Permeability of Phospholipid Monolayers at O/W Interface

Adsorption and formation of monolayers at the o/w interface of phospholipids from organic bulk phase can be followed by measuring the ac impedance(capacitance) of the interface under the control of the potential difference across the interface. The monolayers of six L-α-phosphatidylcholines, dilauryl-(DL-), dimiristoyl-(DM-), dipalmitoyl-(DP-), distearoyl-(DS-), diarachidoyl-(DA-), and dibehenoylphosphatidylcholine(DBPC) have been studied. The presence of the monolayer at the interface was indicated by decreased electric capacitance of the interface; relatively small decrease for DLPC and DMPC and larger decrease for DSPC, DAPC and DBPC in their capacitance in the temperature range between 5 and 30°C, indicating that the DLPC and DMPC monolayers are in a liquid-expanded phase whereas the DSPC, DAPC and DBPC monolayers in a liquid-condensed phase in this temperature range. On the other hand, the monolayer of DPPC exhibits, as indicated by the temperature-dependent change of the capacitance of the interface, a temperature-induced phase transition from the liquid-condensed to liquid-expanded state at 13°C in the same temperature range. Furthermore, kinetics of ion (tetramethyl- and tetraethylammonium ions) transfer at the monolayer-formed o/w interface was studied. The monolayers in the liquid-condensed state reduce the rate of ion transfer for both ions. In contrast, the monolayers in the liquid-expanded state accelerate the transfer of the ions. The results indicate that a phosphatidylcholine monolayer in the liquid-condensed state exerts a hydrodynamic friction on transferring ions, whereas a monolayer in the liquid-expanded state is transparent to the ion transfer. Effect of the double layer structure on the kinetic parameters of ion transfer has been addressed. Phase behavior and ion (tetraethylammonium and perchlorate ions) permeability of dilauroylphosphatidylethanolamine (DLPE) monolayers formed at the o/w interface have also been studied by measuring ac impedance of the interface. Interaction between the charge of transferring ions and that of the monolayer was indicated. Further study would be useful to elucidate the elementary step of ion transfer across the lipid-bilayer membrane/solution interface. [23]

B. Study of Hydrolysis Kinetics of Phospholipid Monolayer by Phospholipase D

Phospolipase D(present in w-phase) catalyzes the conversion of DPPC in the monolayer at o/w interface to L-α-dipalmitoylphosphatidic acid (DPPA), leading to a drastic decrease in the capacitance of the interface. This change in the capacitance is sensitive enough to monitor the course of the enzymatic hydrolysis of the phospholipid monolyers to phosphatidic acid. The rate of the hydrolysis was found to be dependent on the potential drop across the interface; markedly for the enzymes from *Streptomuces* spp. and less markedly for those from plants. Also, the calcium ion requirement was appreciable for the enzyme from *S. chromofuscus* but little for that from peanut. The

parabolic dependence of the hydrolysis rate on the potential drop across the interface was interpreted in terms of Frumkin two-parallel-plate condenser model for the adsorption of phospholipase D. [24, 25]

5. Conclusion

There are number of other voltammetric research subjects than those discussed above that are concerned with most fundamental processes in many area of chemistry, biology and engineering as well as of analytical applications. [1, 26-28] Also, it should be mentioned, spectroelectrochemical approach to ion transfer reactions across the polarizable o/w interface is interesting. [29,30] Finally, electron-transfer voltammetry at the polarizable o/w interface appears interesting and promising. [31]

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