nickel can also be determined using a copper anode. Conditions are similar to those for nickel except that the applied potentials must be 200 mV more positive than for nickel. The anodic reaction rate constants with copper are typically somewhat greater than with nickel, however the applied potential results in increased background noise so that copper offers no signal-to-noise advantage over nickel.

When the background electrolyte contains nickel as suspended nickel hydroxide as described above, copper, nickel, cobalt, silver, platinum, and gold electrodes yield similar analytical currents. These results indicate that suspended nickel hydroxide adsorbs to the metal electrode surfaces and essentially converts them to nickel oxide electrodes.

Silver iodide in its room-temperature crystal form is sufficiently conducting so that it can carry the currents necessary for analytical amperometry. The electrode material is contacted using silver epoxy. The cathodic electrode mechanism involved generation of iodide at the silver:silver iodide interface yielding a current proportional to the rate of oxidation of iodide ions at the silver iodide:solution interface. Hypochlorous acid at sub-parts-per-million level thus yields cathodic currents for pH 6 solutions whereas pH 3 must be used for cathodic amperometry of monochloramine [4]. Accordingly a direct, linear response analytical technique for concentrations from more than 5 mg chlorine per liter down to about 10 $\mu$g chlorine per liter was developed for either monochloramine or the sum of hypochlorous acid plus monochloramine. The concentrations determined were sufficiently low to allow determination of the rate constant for monochloramine formation under realistic water treatment concentration, pH, and ionic strength conditions. The rate constant obtained was $3.2\times10^6$ L/mol/s, in agreement with earlier reported values extrapolated from higher concentrations and less moderate pH solutions.

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# *Adsorptive Stripping Voltammetry—A New Electroanalytical Avenue for Trace Analysis*

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Stripping voltammetry is a powerful electroanalytical technique for trace metal measurements [1]. However, conventional stripping measurements are limited to about 25 metals that electrolytically deposit and/or form an amalgam with mercury. Hence, alternative preconcentration schemes, based on nonfaradaic processes, are desired for extending the scope of stripping voltammetry toward additional analytes.

An extremely useful, sensitive and versatile preconcentration scheme can be achieved via controlled interfacial accumulation of the analyte onto the surface of the working electrode [2]. The voltammetric response of the surface-confined species is directly related to its surface concentration, with the adsorption isotherm providing the relationship between the surface and bulk concentrations of the adsorbate. The most frequently used isotherm is that of Langmuir.

The surface-active characteristics of numerous organic analytes (that commonly complicate their conventional voltammetric measurements) can be exploited for obtaining effective adsorptive accumulation. Trace levels of reducible and oxidizable compounds, such as cardiac glycosides, tetracyclines, phenothiazines, riboflavin, streptomycin, bilirubin, diazepam, tricyclic antidepressants, or mitomycin C, can thus be determined at mercury and carbon electrodes. (Using carbon paste electrodes, both adsorption and extraction occur simultaneously.) Figure 1 illustrates the inherent sensitivity of differential pulse adsorptive stripping voltammetry, as applied to measurement of $5\times10^{-9}$ mol/L digoxin. In addition to low molecular weight compounds, large biological macromolecules, e.g., cytochrome C, chlorophyll, fer-

ritin, or DNA can also be measured. In such cases, the adsorptive approach results not only in enhanced sensitivity, but also a more favorable interaction between the electrode and the redox center of the molecule (due to conformational changes), and hence with enhanced reversibility.

In addition to organic analytes, the formation and interfacial accumulation of appropriate surface-active metal complexes onto the hanging mercury drop electrode permit trace measurement of additional metals. Figure 2 illustrates the steps involved in such measurements of metal ions. In particular, the unique voltammetric and interfacial behaviors of metal chelates of dihydroxyazo dyes allow convenient trace measurement of titanium, thorium, aluminum, iron, uranium, manganese, yttrium, or gallium. Other chelators, e.g., dimethylglyoxime, catechol, oxine, tropolone, or cresolphthalexon, are useful for trace measurements of nickel, vanadium, molybdenum, tin or lanthanum, respectively. Overall, this activity resulted in procedures for measuring more than 25 trace metals; coupled with conventional stripping schemes, about 45 elements are now measurable by stripping analysis. Simultaneous measurement of 2–3 metals is possible, based on an appropriate separation of the metal-chelate peak potentials. Because of its fundamentally different detection principles, the metal chelate approach provides different speciation information compared to conventional stripping measurements, with the fraction of metal measured including the free ion and metals displaced from natural complexes during the formation of the strong adsorbable chelate. When the chelating ligand is present in large concentration excess compared to natural ligands, and a very strong chelate is formed, the *total* metal content is determined. The different nature of the response results in improved performance for metals, e.g., tin or gallium, measurable also by conventional stripping schemes, because interferences (e.g., overlapping peaks, intermetallic compounds) are minimized. Short preconcentration periods result in detection limits as low as $10^{-10}-10^{-11}$ mol/L. Lower levels, e.g., $10^{-12}$ mol/L platinum, can be achieved upon coupling with catalytic reactions (i.e., controlled adsorptive accumulation of the catalyst). Such dual amplification is expected to play an increasing role for ultratrace measurements of species exhibiting adsorption-dependent hydrogen catalytic processes. At low analyte levels ($10^{-7}-10^{-10}$ mol/L), for which the method is usually applied, a linear adsorption isotherm is obeyed and the response is linear. New strategies, such as the use of permselective electrode coatings or the medium-exchange approach, result in substantial improvements in the selectivity and reproducibility. For example, the medium-exchange approach allows convenient measurement of dopamine in the presence of large excess of ascorbic acid. Interferences due to coadsorbing surfactants can be minimized by covering the electrode with a cellulose acetate film.

Adsorptive stripping voltammetry is now a highly sensitive and rapid technique, applicable to analyses in various fields. Its utilization is expanding rapidly and will continue to do so in the near future.

## Acknowledgments

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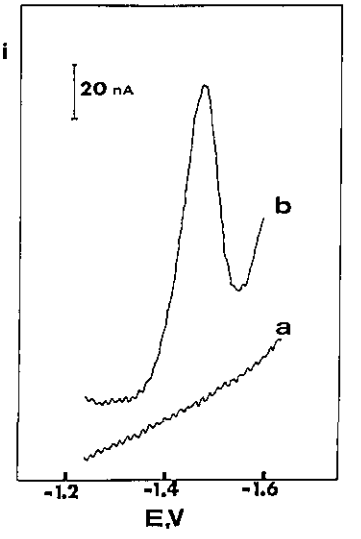


**Figure 1.** Voltammograms for 5 nmol/L digoxin using (a) no and (b) 15-minute accumulation.

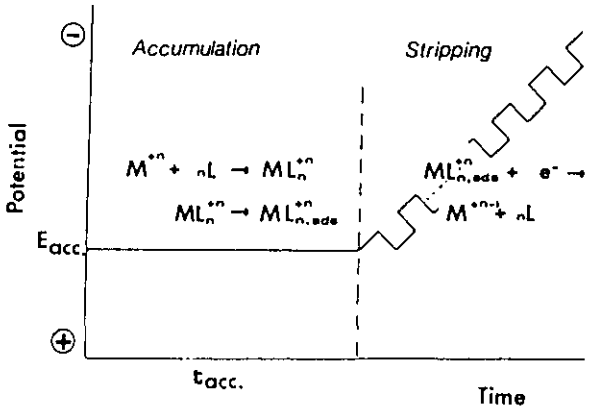


**Figure 2.** Steps in adsorptive stripping measurements of a metal ion based upon the formation, accumulation and reduction of its surface active complex.

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# *Electrochemical Enzyme Immunoassay*

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"Immunoassay" defines the body of techniques which use the antibody (Ab) macromolecule, usually of the IgG class, for the detection and quantitation of an enormous range of simple and complex antigen (Ag) molecules. The success of the methods relies on both the specificity and formation constant of the Ab used, and the ability to detect the interaction between Ab and Ag. Sensitive assays in complex matrices require some kind of label to be present to provide this ability.

The development of immunoassay methods has in large part been driven by the available technology. Thus, although one of the first immunoassays using a label was electrochemically based [1], the state of that art in 1951 was relatively primitive, and could not meet the analytical demands of the Ag. Some years later, Yalow and Berson [2] developed immunoassays based on the radioisotopic label (RIA). For the first time, Ab selectivity was married to very low detection limit technology, and the result was an enormous growth rate in the use of RIA in both the clinical and research laboratory.

RIA has the significant advantage that the label used is not a normal constituent of physiological samples, and interferences of this type are therefore absent. RIA, however, has the disadvantages that accompany radioisotope handling, together with an inability to distinguish label which is bound from that which is not. There has therefore been an enormous effort to find suitable replacements for the radiolabel.

Where the low detection limit of RIA was not required for a successful assay, labels detectable by spectroscopic methods have become important. Where very low detection limits are demanded, then the concept of amplification of, or by, the label has been developed. The most successful of these have been enzyme linked using an immunosorbent phase for Ag extraction (ELISA), although assays based on the lysis of label-containing liposomes are also exhibiting very low detection limits.

In our hands, electrochemically based immunoassays at very low detection limits have also used enzyme amplification and ELISA, with either NAD/NADH and glucose-6-phosphate dehydrogenase [3], or phenyl phosphate/phenol and alkaline phosphatase [4]. The greatest sensitivity thus far has been obtained with ELISA coupled with liquid chromatography with electrochemical detection (LCED) [5].

The power of this approach can be readily illustrated by examining the evolution of its use in an assay for IgG, itself an important analyte. This evolution covers three principal stages of development and a reduction in detection limit of five orders of magnitude.

The basic methodology is shown in figure 1. The assay is essentially a "sandwich" ELISA, using an alkaline phosphatase labelled second Ab for amplification. Oxidative flow amperometry at +875 mV