CATHODIC STRIPPING VOLTAMMETRY AT A HANGING MERCURY DROP ELECTRODE: USE OF POLYAMINOACID FILMS, COPPER(II), DERIVATISATION OF LARGE MOLECULES AND SAMPLE CLEANUP

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Adsorptive/cathodic stripping voltammetric techniques at HMDEs are sensitive techniques based on accumulation of determinand at the electrode before determination. The determinand may be accumulated, for example, directly, or as a derivative (to introduce an electroactive group or to induce adsorption), or as a mercury(I or II) complex (by oxidation of mercury in the presence of the determinand), or as a copper(I or II) complex (copper(I) complexes being formed by reduction of added copper(II) in the presence of the determinand). In this paper some applications of direct determinations, of the derivatisation of large and small molecules, of determinations as copper complexes, and of the use of polyaminoacid films and of sample cleanup, will be discussed.

The first work in this area carried out at Loughborough by Professor A.A.Barros was a major study, in collaboration with Professor Cabral's group at Porto, of the determination of the synthetic food colouring matters and their partial identification at these nanomolar levels using potential shifts with phosphonium compounds.[1] Applications were made to mixtures of food and cosmetic colours in real samples, namely tablet coatings and

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lipsticks. In the latter case the sensitivity of the method allowed dilution after sample pretreatment, and this dilution eliminated matrix effects. Work has begun on the use of solid phase extraction cartridges for sample pretreatment and this should prove advantageous in applying adsorptive stripping voltammetry.

Tyrosine and histidine have been determined after derivatisation with diazotised sulphanilic acid.[2] In studying the possibility of using diazotised amino-tetrazole as an alternative reagent, copper(II) was observed to accumulate as its complex with diazotised amino-tetrazole, but not with amino-tetrazole.[3] This lead to a method for determining copper(II) and, further, to a study resulting in a method of determining histidine as its copper complex,[4] and to a study of the adsorptive stripping voltammetric behaviour of copper complexes of the parent imidazole molecule.[5]

The two pK_a values for the imidazolium ion are 7.1 and between 14.2 and 14.6: the latter value refers to the removal of the proton from the -NH- group in the ring.[6] If the imidazolium ion is represented as H_2Im^+ to show the -NH- hydrogen, then $Cu(II)(HIm)_4^{2+}$ is known[7], as also are polymeric copper(I and II) complexes with Im^- bridging the copper ions.[8] Cyclic voltammograms of accumulated species from the copper(II)-imidazole system (pH=8.5) are highly dependent on the accumulation potential and the imidazole:copper ratio in solution. At 0V vs Ag/AgCl accumulation from $3x10^{-7}$M copper(II) only occurs at $>1x10^{-3}$M imidazole (E_p= -0.46V): this we suggest is accumulation of $Cu(II)(HIm)_4^{2+}$. At E_p= -0.1V and lower concentrations of imidazole, the major peak is at -0.35V. As the

imidazole concentration is increased, the compound responsible for this peak is converted into that responsible for the peak at -0.46V. The reverse process occurs if the copper concentration is increased. The conversion to the compound responsible for the peak at the lower potential also occurs at lower concentrations of imidazole when multiple scans are made between -0.1V and -0.6V. Accumulation at -0.6V gives only the -0.36V peak: the potential of this peak is highly dependent on the imidazole: copper ratio, being at much lower potentials at the lower ratios. Cathodic peaks on the reverse scan are associated with both peaks. The peak at the lower potential is believed to be due to accumulation of the polymeric complex, but this needs confirmation.

Preliminary studies (unpublished) have been made of the adsorptive stripping voltammetric behaviour of the copper complexes of glycylglycylhistidine (ggh) and of glycylhistidylglycine (ghg). In the case of the copper-ggh system (pH 8.4), accumulation at 0V gives a peak at -0.6V: when further scans are then made between 0V and -0.8V this peak grows but another peak appears and grows at -0.37V. An anodic peak at -0.24V seems to be associated with the peak at -0.37V. No peaks are observed when cycling between 0V and -0.4V. Both peaks appear and are unchanged on multiple cycling (-0.8V to 0V) when accumulation is effected at -0.8V: on multiple cycling another peak appears at -0.17V. On accumulation at -0.6V but halting for 1 min at 0V on cycling, the peak at -0.37V is lost, and the second peak is reduced somewhat in height. At this pH ggh is known to form a 1:1 complex involving coordination through the amino-nitrogen and the two amide nitrogens on the glycyl groups, and the 'pyridine-like' nitrogen on the imidazole ring.[9]

No peak is observed for the ghg system when accumulating at 0V and cycling up to -0.9V. A peak begins to show at -0.40V when E_{acc}= -0.1V: this peak is very large at E_{acc} more negative than about -0.25V. The peak height increases with increasing accumulation time at -0.7V up to at least two minutes when scanning from -0.7V to -0.1V and back. When multiple scans are made between -0.7V and -0.1V, after a 2 minute accumulation at -0.7V, large decreases in peak height occur. Ghg is known to be trichelating using the amino-nitrogen and the amide nitrogen on this same glycyl group, and the 'pyridine-like' imidazole-nitrogen.[9,10] Further work is required to elucidate these electrochemical mechanisms, which could be of great significance in bioinorganic chemistry. Ggh, for example, is used to mimic one end of human and bovine albumin chains, in which a histidine moiety is the third from the end.[11]

Preliminary studies have also been made of the use of polyaminoacid films.[12,13] Copper(II) can be accumulated from solutions containing polyhistidine or on a preformed polyhistidine film.[12] Hexacyanoferrate(III) accumulates with copper(II) at a polylysine film: it also accumulates with copper(II) at a bare mercury surface, but in this case the largest peak is obtained for a 3:2 ratio of copper:hexacyanoferrate(III), excess of either progressively reducing the peak height.[13]

We reported previously[14] that bovine serum albumin labelled by means of fluorescein isothiocyanate or rhodamine B isothiocyanate gives adsorptive stripping voltammetric signals characteristic of the dye moiety and also of sulphide. At that time we thought that the labelling reagents themselves also gave

the sulphide peak and, when present in the molecule, the dye moiety peak. This is not always the case.[15] Aqueous solutions of the reagents degrade extensively in tens of minutes, and then give the sulphide peak. Freshly prepared fluorescein isothiocyanate only gives the sulphide peak at accumulation potentials more negative than 0V: freshly prepared phenylisothiocyanate does not give the sulphide peak at any accumulation potential.

The formation of these thiocarbamoyl derivatives of proteins is the first step in the Edman degradation used for sequencing proteins.[16] Treatment with acid removes the end amino acid with the label in a cyclisation reaction to form the thiazolidinone derivative. Further treatment with acid causes a rearrangement to the more stable thiohydantoin derivative. Adsorptive stripping voltammetry of the phenylthiohydantoin derivative of tyrosine and the methylthiohydantoin derivative of glycine gave peaks which we considered to be due to mercury salt formation.[15] In the presence of copper(II), however, the thiohydantoins give peaks due to accumulation of their copper complexes.[17] The glycine derivatives uniquely, at E_{acc}= -0.1V, give stripping peaks at much more negative potentials. Oxidative combination of two molecules of thiohydantoin derivatives of glycine has been reported,[18,19] and the unusual peak obtained here may be associated with the copper complex of the compound formed in this process.

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ADSORPTIVE STRIPPING VOLTAMMETRY: ADSORPTION OF DIOXIME LIGANDS AND COBALT AND NICKEL COMPLEXES AT MERCURY THIN-FILM ELECTRODES

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The pre-concentration step in adsorptive stripping voltammetry involves adsorption accumulation of metal ion complexes of low solubility at the electrode surface, generally mercury, at a small negative applied potential in order to ensure maximum adsorption. In the case of cobalt and nickel, dioxime ligands form sufficiently specific complexes for their identification and determination. After adsorption accumulation, the adsorbed metal ion complexes are reduced by a negative scan to the zero oxidation state resulting in a current peak, the height of which is proportional to concentration.

We have developed a procedure for quantification of cobalt and nickel in flowing solution at the wall-jet mercury thin-film electrode, using nioxime (1,2-cyclohexanedioxime) ligand in a biological buffer, HEPES (pK_a 7.5) [1]. The optimised experimental procedure involves, after formation of the mercury film in acidic nitrate medium, adsorption accumulation of the complexes at -0.7V vs. Ag/AgCl in 30mM HEPES/0.1M $NaClO_4$ at pH 7.6 followed by a differential pulse negative scan from -0.8V. The nickel peak appears at -0.93V and the cobalt peak at -1.01V, there being catalytic enhancement of the cobalt current peak. This method works well in the nanomolar concentration range - at higher concentrations the mercury surface rapidly becomes saturated with complex.

Investigations into the nature of the adsorption and reduction processes were carried out using a rotating disc mercury thin-film electrode (MTFE) formed on a glassy carbon substrate of geometric area $0.4cm^2$, and dioxime ligands in the same 30mM HEPES buffer/0.1M $NaClO_4$ medium by impedance and linear sweep voltammetry. Experiments focussed particularly on nioxime ligands. Previous work at the hanging mercury

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