Cathodic Stripping Voltammetric Determination of Sulfonamides as Copper(I) Complexes at a Hanging Mercury Drop Electrode

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Sulfonamides that form stable metal complexes can be accumulated at a hanging mercury drop electrode in the presence of copper(II). In this preliminary study sulfadimidine, sulfathiazole, sulfamerazine, sulfadiazine and sulfapyridine, which have an ortho heterocyclic nitrogen atom on the ring attached to the sulfonamide nitrogen atom, and which are known to form stable complexes with copper(II), have been accumulated in this way and have been determined indirectly by reduction of the complexed copper to copper amalgam. Sulfanilamide, sulfaguanidine and sulfacetamide, which do not form stable complexes, are not accumulated. Preliminary results indicate that the sulfonamides are accumulated as their copper(I) complexes

Bult has reviewed the use of metal complexes of sulfonamides in pharmaceutical analysis and in drug therapy.[1] Sulfonamide drugs are soluble in both acidic and alkaline solution owing to the presence in their structures of the aromatic amine and the sulfonamide groups, respectively. Many sulfonamide drugs are prescribed as sodium salts, the acidic hydrogen in the sulfonamide group being displaced to give a water soluble drug. In forming metal complexes this acidic hydrogen is lost in competition with the metal ion, allowing the sulfonamide nitrogen to serve as a donor atom in the complex. Stable complexes are reported to be formed, however, only by those sulfonamides whose structures contain a heterocyclic nitrogen atom ortho to the sulfonamide group. Bult and co-workers[2] prepared and studied a range of copper(II)–sulfonamide complexes. Sulfadiazine, sulfamerazine, sulfamethoxydiazine, sulfamethoxypyridazine, sulfapyridine and sulfathiazole were shown to form dimeric complexes of the type Cu_2L_4, in which the sulfonamide and the heterocyclic nitrogen atoms in each sulfonamide molecule are coordinated to two different copper ions. (A bidentate, mononuclear complex would have four-membered rings.) Sulfonamides with a substituent on the other carbon atom in the heterocyclic ring that is ortho to the heterocyclic nitrogen atom, *e.g.*, sulfadimethoxine, sulfadimidine and sulfasomidine, on the other hand, gave complexes classified as monomeric or polymeric. This modified behaviour was reported to be caused by steric hindrance by this substituent.[2]

Argentimetric methods have been reported for the determination of those sulfonamides which form insoluble silver salts: these are mainly the ones that form stable copper complexes.[1,3] The characteristic colours given by individual sulfonamide drugs in the presence of copper(II) are used for identification purposes.[4]

Cathodic stripping voltammetry at a hanging mercury drop electrode (HMDE) has been used extensively as a trace technique. Suitable organic compounds can be accumulated by adsorption on the HMDE and then determined by reduction.[5] Trace metals can be adsorbed as metal complexes and can then be determined directly, by reducing the metal ion, or indirectly, by reducing the ligand.[6] Organic compounds that are not reducible, but which can complex metals, can be determined indirectly in a similar way by adsorbing a metal complex and then reducing the metal ion. To date, however, faradaic accumulation steps have been used for this purpose, mercury (I or II) complexes being produced during accumulation by oxidation of mercury metal, or the copper(I) complex being formed during accumulation by reduction of copper(II).[7]

Previous work has shown that many sulfonamides form stable copper(II) complexes.[1,2] Preliminary work is reported here that indicates that those sulfonamides that form stable copper(II) complexes can be accumulated as (stable) copper(I) complexes at a HMDE, and can then be determined by cathodic stripping voltammetry.

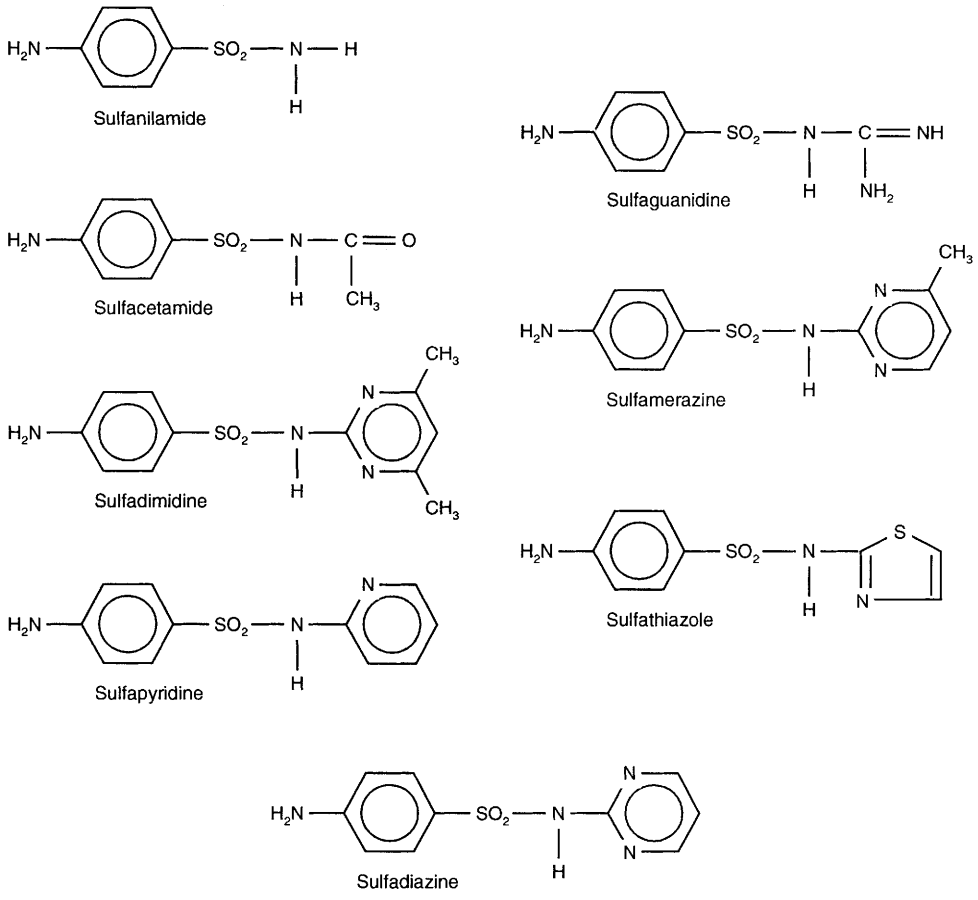
Experimental

Apparatus and Reagents

Cathodic stripping voltammetry was carried out with a Metrohm 646/647 VA Processor, using a multi-mode electrode in the HMDE mode. The three-electrode system was completed by means of a glassy carbon auxiliary electrode and an Ag/AgCl (3 mol dm^{-3} KCl) reference electrode. All potentials are quoted relative to this reference electrode. Differential-pulse voltammetry was carried out with a pulse amplitude of 50 mV, a scan rate of 10 mV s^{-1} and a pulse interval of 1 s.

The standard copper(II) solutions were prepared by diluting a BDH (Merck Ltd., Poole, Dorset, UK) Spectrosol copper(II) nitrate standard solution. Samples of the sulfonamides used (structures given) were obtained from Sigma and other sources. Standard solutions were made by dissolving weighed amounts of the sulfonamides in a small volume of dilute sodium hydroxide solution before making up to volume.

The general procedure used to obtain cathodic stripping voltammograms was as follows: a 20-ml aliquot of buffer solution (phosphate buffer 0.1 mol dm^{-3}) was placed in a voltammetric cell and the required volumes of sulfonamide and copper(II) solutions were added using a micropipette. The stirrer was switched on and the solution was purged with nitrogen gas for 5 min. After forming a new HMDE, accumulation was effected for the required time at the appropriate potential whilst stirring the solution. Medium drop size (approximately 0.40 mm^{-2}) was used. At the end of the accumulation time the stirrer was switched off, and after 10 s had elapsed to allow the solution to become quiescent, the negative-going potential scan was initiated. When further volumes of sulfonamide or copper(II) solution were added to the cell, the solution was deoxygenated with nitrogen gas for 1 min before carrying out further voltammetry.



Results and Discussion

Typical differential-pulse stripping voltammograms of sulfonamides obtained after accumulation at −0.1 V in pH 6.0 Britton–Robinson buffer in the presence of increasing concentrations of copper(II) are shown in Fig. 1. Sulfadimidine, which forms a stable copper(II) complex, is seen to be accumulated strongly in the presence of copper(II), the extent of accumulation increasing with increasing copper(II) concentration in the range shown, whereas sulfacetamide, which does not form a stable copper(II) complex, is not accumulated in this way. Other sulfonamides that have been found to be accumulated are sulfathiazole, sulfamerazine, sulfadiazine and sulfapyridine. Others that have been found not to be accumulated are sulfaguanidine and sulfanilamide. This behaviour can be anticipated from the complexing properties, or lack of them, of the individual sulfonamides.

The optimum pH for accumulating the sulfonamides was found to be 6.0. At pH <4.0 virtually no accumulation is observed; at pH >6.0 accumulation with a good stripping peak is observed up to pH 11, but the peak height decreases progressively with increasing pH. Calibration graphs for the determination of sulfadimidine at pH 6.0 (phosphate buffer) with 5×10^{-7} mol dm^{-3} copper(II) solution and with 2 min accumulation were rectilinear from 1×10^{-8}–1.5×10^{-7} mol dm^{-3}; the detection limit was about 1×10^{-9} mol dm^{-3}. The sensitivity is approximately doubled by using phosphate buffer in place of Britton–Robinson buffer.

Using an accumulation potential of −0.1 V in pH 6 Britton–Robinson buffer, with an accumulation time of 2 min, the peak potentials obtained for the differential-pulse CSV of sulfadimidine, sulfapyridine, sulfathiazole, sulfamerazine and sulfadiazine were in the range from −0.23 to −0.25 V, and the peak currents were 48, 28, 40, 28 and 22 nA, respectively. The peak obtained with sulfadiazine, which was the smallest of those obtained with the sulfonamides that are accumulated, was not so well defined as the others in terms of separation from the background.

The effect of accumulation time was studied. Accumulation increased with increasing accumulation time, under the conditions used here, up to about 2 min. Beyond this time the peaks began to split, with a second, sharper peak appearing at more negative potentials, the original peak decreasing markedly in size. This was probably due to the complete formation, at the sulfonamide concentration used (1×10^{-7} mol dm^{-3}), of a monolayer of adsorbate after 2 min, with formation of multilayers of adsorbate with different stripping characteristics at longer accumulation times. The original peak for sulfathiazole, for example, is at −0.25 V, whereas the new sharper peak is at −0.40 V.

A preliminary study has indicated that the accumulated complexes are copper(I) complexes. The main evidence for this is that when accumulation is carried out on open circuit, with the working electrode disconnected, no accumulation peaks are observed on scanning the potential. Further, when accumulation is carried out at 0 V the peaks are much smaller or are absent.

A fuller fundamental investigation is being made of the CSV of a wide range of sulfonamides with different structures and complexing abilities, in order to assess the effect of the structure of the sulfonamides on the ability to form and accumulate the copper(I) complexes. Further, there is a need for rapid and reliable screening procedures for detecting veterinary sulfonamide drug residues in meat down to at least the 10 μg kg^{-1} level.[8] The five sulfonamides shown here to accumulate as copper(I) complexes, plus sulfaquinoxaline (not yet studied), are the most commonly used sulfonamides in large-animal farming in the UK, although other sulfonamides

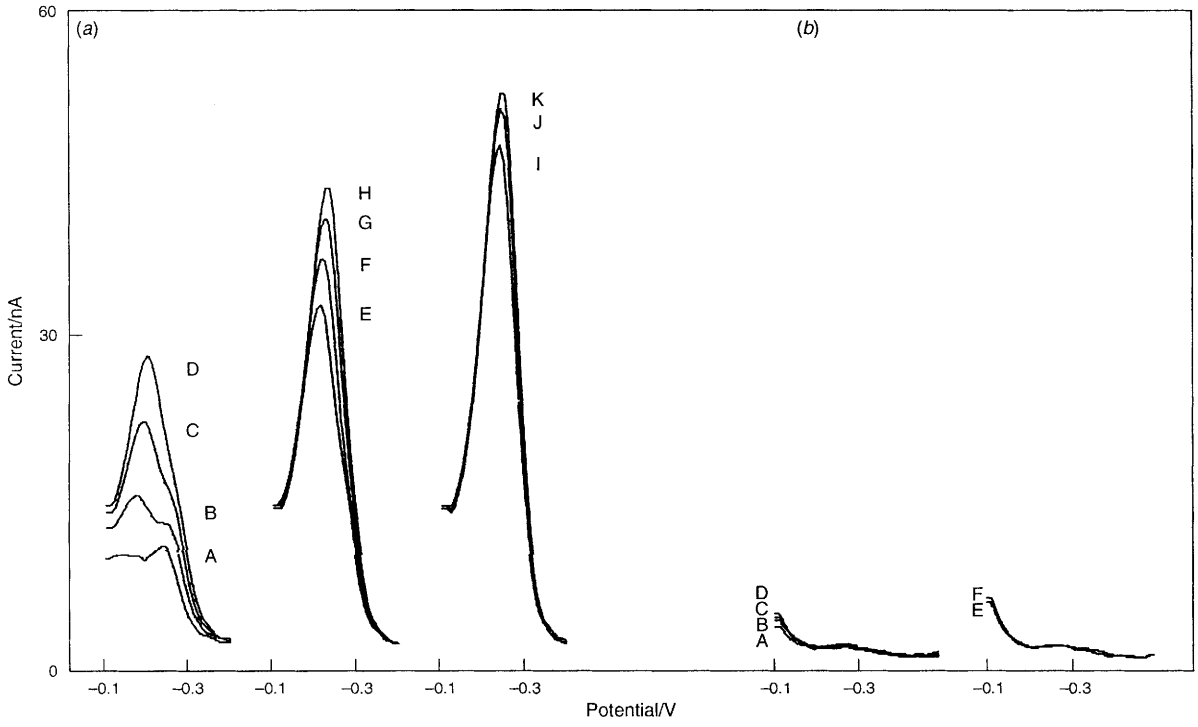


Fig. 1 Typical differential-pulse cathodic stripping voltammograms showing the effect of copper(II) concentration on the accumulation of (*a*) sulfadimidine and (*b*) sulfacetamide. pH = 6.0 (Britton–Robinson buffer). Accumulation potential = 0.1 V. Accumulation time = 2 min. Sulfonamide concentration = 2.0×10^{-7} mol dm^{-3}. Copper(II) concentration for both *a* and *b*; A, 0; B, 0.5; C, 1; D, 1.5; E, 2; F, 2.5; G (*a* only), 3; H (*a* only), 3.5; I (*a* only), 4; J (*a* only), 4.5; and K (*a* only), 5×10^{-7} mol dm^{-3}

are used worldwide.[8] Clearly, the method being developed here, using a mercury electrode, is unsuitable for use in abattoirs, and our intention is to attempt to convert this method for use with solid electrodes, preferably in a disposable sensor device, with an acceptable detection limit.

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