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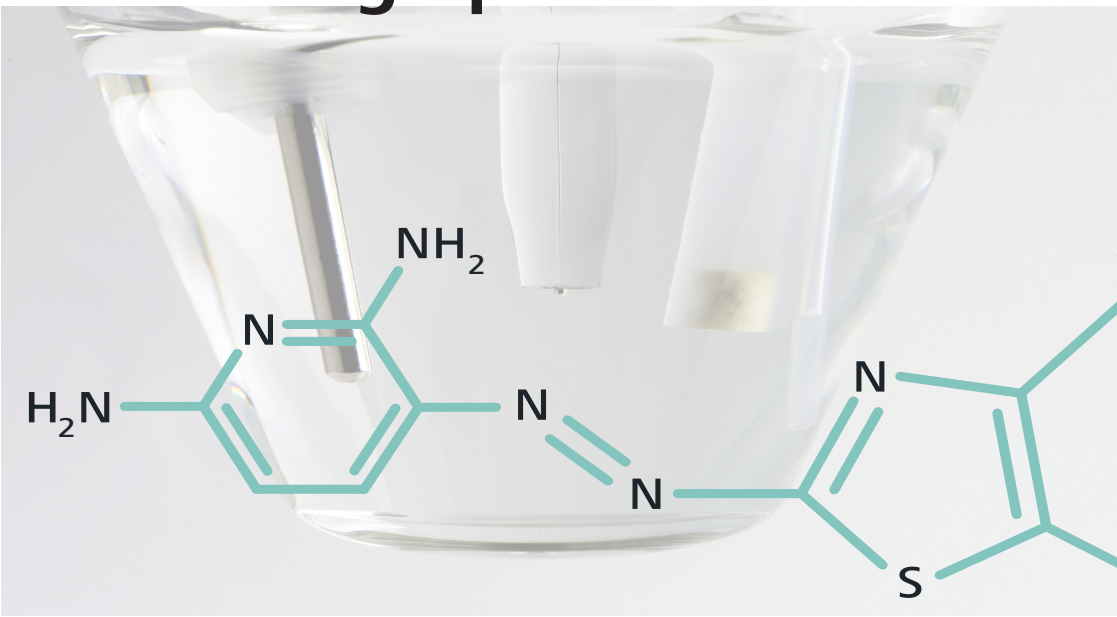

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by W. FRANKLIN SMYTH* and J.S. BURMICZ**

Introduction

The technique of anodic stripping voltammetry (ASV) for the trace analysis of metal ions such as Cu(II), Pb(II), Cd(II), Zn(II) etc. has received considerable attention in recent years[1,2]. ASV is based on controlled electrolytic accumulation of the metal ion at indicator electrodes such as the hanging mercury drop (HMDE) or mercury film electrode (MFE) prior to its determination by electrolytically stripping the accumulated species back into solution by imposition of a potential scan in the anodic/positive direction. ASV has primarily been used for the determination of ppb and sub-ppb concentrations of toxic heavy metal ions in a variety of sample matrices from natural waters to body fluids, such as whole blood.

Organometallic molecules such as those involving tin as the central metal atom can be determined by ASV[3] following liberation of the metal from the complex or by Anodic Stripping Voltammetry of the intact complex. Woggon[3] has studied the influence of plating potential and time, the volume of the Hg mercury drop and temperature on the ASV of $RSnCl_3$ (where R = butyl or octyl), $C_6H_5\ SnCl_2\ CH_3$, $SnCl_4$ and $(C_6H_5CH_2)Sn(O)OH$ and optimised conditions for their analysis in fungicide residues. Booth and Fleet[4] have applied ASV to the stripping of organotin molecules as free radicals which can be stabilised by adsorption on mercury electrodes, following their formation by a one electron reduction process.

The technique of cathodic stripping voltammetry (CSV) has the deposition step carried out electrolytically at anodic potentials and electrolytic stripping by imposition of a cathodic voltage scan. This method has been applied to the determination of a range of inorganic anionic species such as Cl^-, Br^-, I^-, CrO_4^{2-}, MoO_4^{2-}, VO_3^-, WO_4^{2-} etc.[5,6] in addition to organic anions such as dithizonate, diethyldithiophosphate, oxalate and succinate[7]. Dithiocarbamate pesticides[8], organosulphur drugs[9] and other organic molecules can also be determined by this CSV technique in which the organic entity forms a partially insoluble compound/complex with mercury which deposits at the electrode surface at anodic potentials. Limits of detection can be lowered by several orders of magnitude when the CSV technique is compared to conventional polarographic techniques such as differential pulse polarography (DPP), square wave polarography (SWP) etc. as is illustrated in Table 1.

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Table 1: Selected Organic Molecules that can be determined by CSV

Molecule	Pretreatment / Supporting Electrolyte	Limit of Detection	Ref.
Dithiocarbamates	Aqueous solution	10^{-7}M	8
Thioamides	Biological fluids	2×10^{-8}M	9
Selenocystine, cystine, cysteine	Dilute acidic solution	Cysteine 10^{-9}M Cystine 10^{-8}M	10
Nucleic acid bases	Borate buffer	10^{-8}M	11
Penicillins	Alkaline hydrolysis to corresponding penicilloic acid, deposition as copper complex of penicillamine, stripping at –0.40 V	2×10^{-10}M	12
2-Thiobarbiturates	Aqueous solution	5×10^{-8}M	13
2-Mercaptopyridine N-oxide	Aqueous solution	8×10^{-10}M	14
Thiourea	Aqueous solution	2.5 ngL^{-1}	15
Ethyl xanthate	In a sulphide mineral flotation plant	10^{-8}M	16
5-Fluorouracil	Borax-$HClO_4$ buffer pH 7.8	10^{-8}M	17
Purine (cathodic stripping voltammetry at the hanging copper amalgam electrode)	0.1M $LiClO_4$ pH 2	5.10^{-9}M	18

The 1980's have seen an increasing interest in organic stripping measurements for species that cannot be accumulated by electrolysis[19,20]. In particular, the technique of adsorptive stripping voltammetry (AdSV) refers to an electroanalytical technique in which the analyte is preconcentrated onto the electrode surface by adsorption, followed by voltammetric determination of the surface-active species. This has resulted in the determination of many organic molecules, possessing surface activity, at the nanomolar concentration level. Detection limits have been quoted in the 10^{-10} ... 10^{-11}M region and are thus comparable to the sub-ppb detection limits quoted for the ASV determination of toxic heavy metal ions such as Pb(II), Cu(II), Cd(II), Zn(II) at the mercury film electrode (MFE). If the molecule is electroinactive (tensides, alkaloids), the corresponding tensammetric peak is obtained on the voltammetric scan[21] – in these cases, detection limits are somewhat higher at ca. 10^{-6} M. Recent publications as listed in Table 2, have shown that AdSV can be used in trace determinations of drugs (e.g. tranquillisers, antibiotics, antiulcer and cardiac agents), pesticides (e.g. thiourea, nitro- and triazine-containing molecules) and naturally occuring molecules (e.g. DNA, progesterone, testosterone, dopamine, riboflavin). The technique is rendered selective in complex matrices such as blood, urine, cattle feed etc. by using medium exchange as a batch or particularly as an on-line process. In this latter case, the link-up of flow injection analysis and AdSV should be referred to for the direct assay of chlorpromazine in urine[32] and doxorubicin/adriamycin also in urine[33].

Theory and Practice of Stripping Techniques

CSV (Cathodic Stripping Voltammetry)

Brainina[5] has reviewed the theory and applications of film stripping voltammetry for the determination of anions. The relevant electrode reactions are, for a mercury indicator electrode:

deposition step: $2Hg \rightleftharpoons Hg_2^{2+} + 2e$

$Hg_2^{2+} + 2A^- \rightleftharpoons Hg_2A_2 \downarrow$

stripping step: $Hg_2A_2 + 2e^- \rightarrow 2Hg + 2A^-$

where A^- is the organic anion removed from solution (assumed for simplicity to be monovalent) and Hg_2A_2 is the sparingly soluble compound forming the film on the electrode surface. Brainina has also given theoretical consideration to the particular case of irreversible film dissolution. Since in the process of electrodissolving a compound localised on the electrode surface, the metal ions being reduced come into contact with the electrode surface, the case may be regarded mathematically as analogous to dissolving the metal from the inert electrode surface.

A relationship can then be written for irreversible dissolution of a compound from the indicator electrode surface, i.e. the peak or maximum current is given by

$$i_p = 0.37 \frac{\alpha n F}{RT} \cdot V \cdot C_{Hg_2A_2}$$

where V is the scan rate, $C_{Hg_2A_2}$ is the concentration of the sparingly soluble mercury compound and the symbols α, n, F, R, T have their usual significance.

AdSV (Adsorptive Stripping Voltammetry)

In the adsorption of a species at the indicator electrode – solution interface, formation of the adsorbed layer is governed by the rate of diffusion of the species from the bulk of solution to the electrode surface and by the rate of adsorption of the species from the solution layer in direct contact with the indicator electrode surface. In AdSV, the second process is relatively rapid and the overall process is therefore governed by diffusion of the species to the electrode surface.

Kano et al[41] have derived an equation for the peak current i_p of the reduction of adriamycin at an HMDE, assuming diffusion-controlled adsorption and that i_p is proportional to the surface concentration of the drug, Γ:

$$i_p = kA\Gamma = kAC\left[\left(\frac{D}{r}\right)t_{acc} + 2\left(\frac{D}{\pi}\right)^{\frac{1}{2}} t_{acc}^{\frac{1}{2}}\right]$$

where k is a proportionality constant, A is the electrode surface area, C is the adriamycin concentration in solution, D is the diffusion coefficient, r is the HMDE radius and t_{acc} is the accumulation time. At large values of C and/or t_{acc}, i_p approaches a limiting value i_p^{max}, for which it is assumed that

$$i_p^{max} = kA\,\Gamma_m$$

where Γ_m corresponds to surface concentration at complete surface coverage or saturation.

Koryta[42] has shown that $\Gamma_m = 7.36 \times 10^{-4}\, C\, D^{\frac{1}{2}} t_m^{\frac{1}{2}}$ where t_m is the time required for complete electrode coverage and Γ_m the maximum electrode surface coverage in mol cm^{-2} from a bulk concentration C in mol cm^{-3}.

It has been found experimentally that i_p increases linearly with the square root of t_{acc} (ref. 26,43 – 45) assuming less than complete surface coverage and that there is no interaction between the adsorbed molecules on the electrode surface. i_p is also proportional to the product of C and $t_{acc}^{\frac{1}{2}}$ when neither of these values is too large.

It thus follows that the AdSV technique should be employed under conditions where the peak height increases linearly with the concentration of the studied molecule. When this dependence deviates from linearity (especially when i_p approaches a limiting value), the experimental conditions must be modified (solution diluted, t_{acc} shortened or solution not stirred during t_{acc}).

An example typical of this sort of phenomenon is illustrated in Figure 1 below. This is taken from reference[17]. The figure shows the the relationship between the stripping signal i_p and the accumulation (deposition) or pre-concentration time for three different concentration levels of 5-Fluorouracil. While the lines A and B show a good correlation between deposition time and peak height at the $5 \cdot 10^{-7}$M and 10^{-6}M levels, an increase in the solution concentration to $5 \cdot 10^{-6}$M shows a dramatic difference in the response curve. There comes a point at this concentration where the drop itself is saturated with the adsorbed compound. It is clear from this figure that deposition times longer than about 10 seconds will lead to a non-linear time deposition/peak height function.

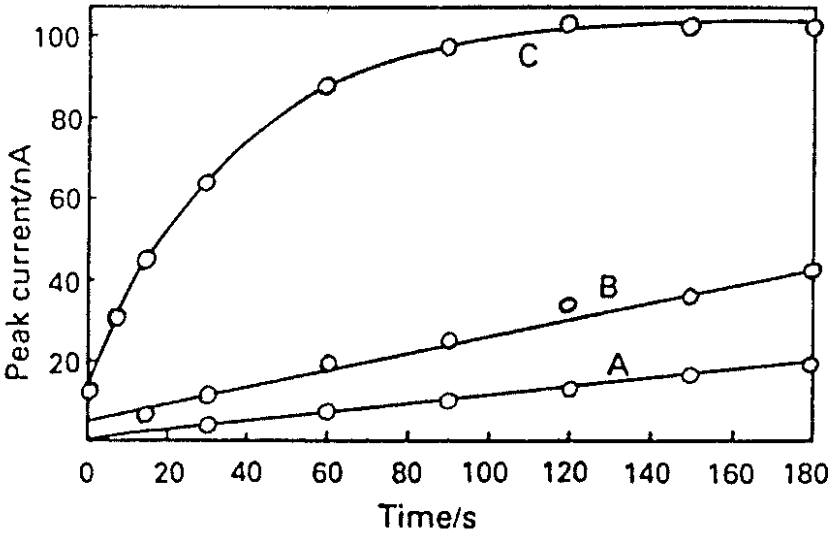

Figure 1 The effect of preconcentration time on the stripping peak current of 5-Flourouracil (pH 7.8). Fluorouracil concentrations: A, 5×10^{-7}M; B, 10^{-6}M, and C, 5×10^{-6}M. (Reproduced with permission.)

This effect is directly reflected in the peak height/concentration relationship. This means that there will always be an optimum range for concentrations of the species in solution for a given deposition time. In fact it can also be said that the determination of certain species is easier in more dilute sample solutions than at higher concentrations. This may be for several reasons. Perhaps the most pertinent is that of linear calibration ranges. Dilute solutions can always be preconcentrated at the electrode surface for a longer period of time; with automated microprocessor based equipment, this poses no problem at all. Preconcentration as such is a function of deposition time and not of any further "wet chemical" stages. There is also a better possibility for greater linear ranges of concentration with more dilute solutions. But perhaps the most relevant point, especially with respect to biological and highly contaminated samples, is that dilution from a ppm to ppb concentration level of analyte means that the matrix effect, i.e. that of the bulk sample, is also diluted. As the dilution may take place with an optimum supporting electrolyte for the species concerned, a certain degree of signal enhancement or optimisation may also be achieved.

The amount of surface active molecules accumulated on the electrode surface is also affected by variables other than bulk concentration and time of adsorption/accumulation such as accumulation potential[46,47], electrolyte composition (ionic strength, pH value, solvent)[46,47], electrode material and temperature. Optimum conditions for maximum accumulation of strongly adsorbing species – in order to achieve maximum sensitivity in the subsequent voltammetric stripping response – are usually found by examining the peak current enhancement at a given accumulation time as compared to that without accumulation using a 10^{-6} – 10^{-7}M solution. Attention must also be paid to reproducibility and a compromise may have to be made between it and the working concentration range.

The hanging mercury drop (HMDE) is widely used for measuring reducible surface active species while carbon paste, wax-impregnated graphite and platinum electrodes are used for oxidisable analytes. A cleaning step can be required with solid electrodes when the species of interest is not desorbed during the voltammetric scan. Using carbon paste electrodes, the adsorptive accumulation of various compounds is accompanied by extraction into the body of the electrode[48].

The stripping step in AdSV can be performed using a variety of voltammetric waveforms such as linear scan[15], differential pulse[22], square wave[26,31] and staircase[26] modes. The differential pulse mode has been widely used because of its corrrection for the charging current and commercial availability. When this mode offers little improvement in sensitivity over the linear scan mode then the latter is preferred due to its speed of operation. Brown and Anson[49] have produced equations for the peak current and potential in differential pulse and linear scan measurements of surface bound species.

Table 2: AdSV of Selected Organic Compounds

Molecule	Indicator Electrode	Supporting Electrolyte	Limit of Detection	Ref.
Riboflavin	SMDE	10^{-3}M NaOH	2.5×10^{-11}M	22
Chlorpromazine and other phenothiazines	Carbon paste, impregnated graphite	Phosphate buffer	5×10^{-9}M	23, 24
Diazepam Nitrazepam	SMDE	Acetate buffer	5×10^{-9}M	25
Cimetidine	SMDE	0.1M HCl	4×10^{-9}M	26
Progesterone and testosterone	SMDE	5×10^{-3}M NaOH	2×10^{-10}M	27
NO_2-containing pesticides	SMDE	Britton-Robinson buffer	5×10^{-10}M	28
Diltiazem	SMDE	5×10^{-2}M NaOH	4×10^{-9}M	29
Camazepam and bromazepam	HMDE	4×10^{-2}M Britton-Robinson buffer pH 5	Camazepam 3.4×10^{-10}M Bromazepam 3.5×10^{-9}M	30
Cephalothin	SMDE	7.5×10^{-2}M $HClO_4$	10^{-8}M	31
3-(4,5 dimethyl-2-thiazolylazo)-2,6-diamino-pyridine	HMDE	Phosphate buffer pH 7	8×10^{-10}M	34
Quinoxaline-N-dioxide derivatives	SMDE	0.1M $NaClO_4$ (5% $^{v}/_{v}$ DMF)	3×10^{-10}M	35
Synthetic colouring matters	HMDE	Buffers of pH 4, 7 and 10	10^{-10}M	36
Streptomycin	SMDE	0.01M NaOH	7×10^{-10}M	37
Triton X-100	SMDE	0.1M NH_3-NH_4Cl	1 μgL^{-1}	38
Folic acid	SMDE	0.1M H_2SO_4	10^{-10}M	39
Flurazepam	SMDE	0.1M acetate buffer	10^{-8}M	40

The stripping step can also be carried out in a simple electrolyte solution as opposed to the original complex matrix in which the preconcentration was carried out[50], thus improving the selectivity of the technique by elimination of electroactive interferences in the complex matrix. This is also accomplished by the use of flow injection analysis (FIA) together with adsorptive stripping voltammetric detection[32]. In general, linear calibration plots are observed in the 10^{-7} to 10^{-10}M region with deviations observed at higher concentrations due to electrode saturation. Because of lower background currents, mercury electrodes offer lower detection limits (10^{-10}...10^{11}M) as compared to solid electrodes (10^{-8} ... 10^{-9}M). Relative standard deviations for replicate measurements at mercury electrodes range from 2 ... 6% and from 5 ... 12% at solid electrodes.

Practical Examples and Applications:

The application of Adsorptive Stripping Voltammetry or AdSV is undoubtedly a very attractive technique. In essence, there are very few techniques so broadly applicable to the analysis of organics, heavy metals and anions. Indeed, the low (trace) level analytical possibilities using a single technique which allows the preconcentration of the analyte from the solution itself without recourse to extra wet chemical extraction stages, not only minimises the time requirement for the analytical routine as a whole, but also limits the possibilities of sample contamination from extra unit processes within the analytical scheme.

The following examples are extracted from the literature cited and show the diversity of applications in day-to-day routine analysis.

The instrumentation used was, in all cases, the METROHM 646 VA Processor and the 647 VA Stand. Where the entry into the memory has been made regarding the sample name or designation, e.g. "Tartrazine" or "Red 6", then the instrument has printed the given name. Where the chemical designation has not been entered, then the potential alone is printed out. This is then related, in the result calculation, to a peak height expressed in current units, which is then referred to a calculated concentration, after dilution factors etc. are taken into account by the instrument. As voltammetry is a non-destructive technique, the possibility for standard addition within the same sample vessel means that only one sample per analysis is required rather than a successive stream of identical samples containing increasing quantities of added standard. The standard additions can either be made manually with a pipette or automatically with a 665 Dosimat in the "dispense" mode.

The first examples are for the compounds shown in figure 2. These structures represent two of a series of 1,4-Benzodiazepines. The structures represent Camazepam and Bromazepam.

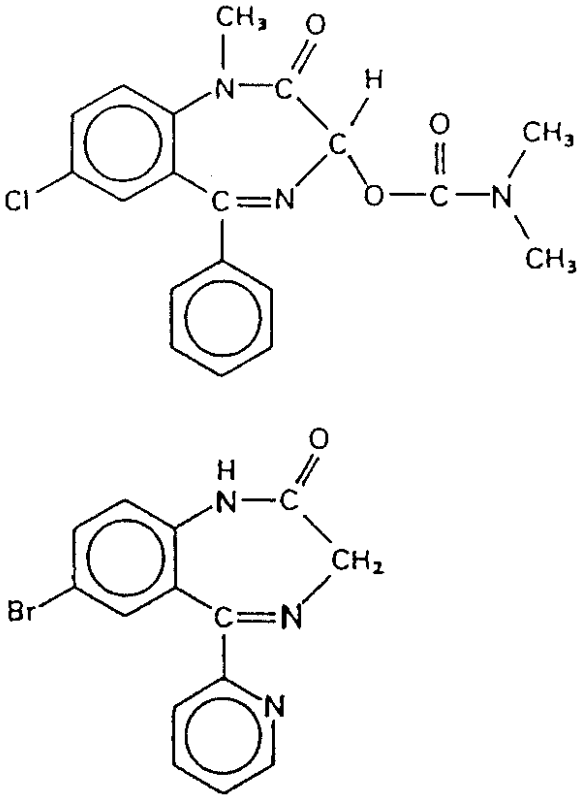

Figure 2 Structures of 7-chloro-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl dimethylcarbamate (Camazepam) and 7-bromo-1,3-dihydro-5-(2-pyridyl)-2*H*-benzodiazepin-2-one (Bromazepam). (Reproduced with permission.)

The resulting stripping voltammograms are shown in figure 3 below. The analytical conditions are also illustrated.

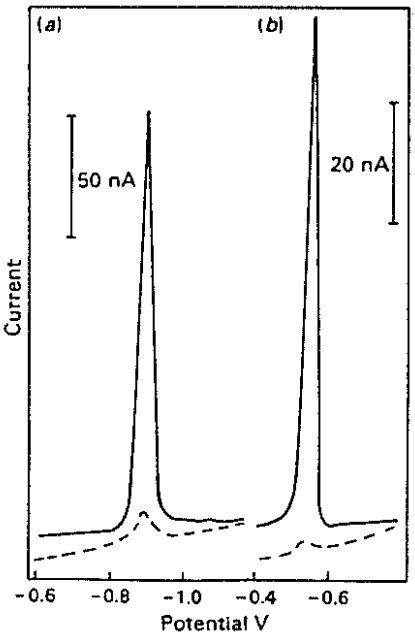

Figure 3 The differential pulse adsorptive stripping voltammograms with stirring at 1920 rpm for (a) 1.29×10^{-8}M Camazepam. 270 s accumulation period at -0.60V (v. Ag-AgCl-3M KCl) in 0.04M acetate buffer, at pH = 5; (b) 6.02×10^{-8}M Bromazepam. 90 s accumulation period at -0.40V (v. Ag-AgCl-3M KCl) in 0.04M Britton-Robinson buffer, pH = 5. Scan rate, -15 mV s^{-1}; pulse amplitude, 70mV. The discontinuous line represents the voltammetric response without accumulation. (Reproduced with permission.)

As mentioned in the text, this technique has also been applied to the analysis of synthetic colouring materials for food products as well as pharmaceutical preparations. The examples shown below give an indication as to typical determinations. Figure 4 shows the linearity of response (standard curve) for the colouring material known as Amaranth. The analytical conditions are also given in the accompanying text. By comparison, figure 5 shows the determination of Tartrazine in a tablet coating. In this case two sets of stripping signals are obtained. The most analytically usable are those occuring at ca -0.85V. Once again the non-destructive nature of this technique allows the use of standard additions for the quantitative calculation of the analyte. Note also that the Tartrazine signal has been optimised by the addition of the signal enhancing material TPPC (tetraphenylphosphonium chloride). As mentioned previously, the need to dilute the sample solution has more than been compensated by the signal enhancing properties of the TPPC and the elimination of matrix effects following dilution with an optimised supporting electrolyte. Tartrazine is also used extensively as a colourant for fruit drinks or squashes (as opposed to fruit juices). It has also been linked to hyperactivity problems in young children.

The last two examples are applications in the pharmaceutical industry (tablet coatings) and the cosmetic industry (lipstick colourants). The materials concerned are Amaranth (in tablet coatings) shown in Figure 6 as well as Lithol Rubine and Lake Red C (in lipstick), shown in Figure 7. The analytical conditions are also illustrated briefly in the associated text. Figure 7 is particularly interesting as it allows the separation of the two components in the lipstick. The effect of pH on the resulting stripping signals is also quite clear. This again reinforces the idea of matrix effect elimination, or matrix optimisation using sample dilution with a selected buffer system. This enables enhanced resolution of the two components. For a more detailed description of the experimental details (such as sample preparation, and so on), the reader is directed to reference 36, which contains an in-depth investigation for sixteen food and two cosmetic colouring matters. All of these materials are determined at the Hanging Mercury Drop Electrode (the HMDE or in the case of the METROHM VA Stand 647, the MME).

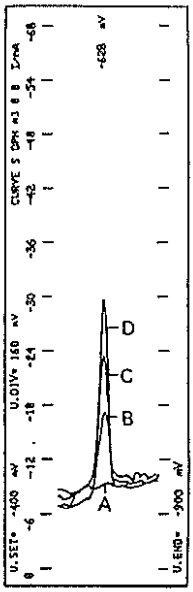

Figure 4
Typical differential pulse adsorptive stripping voltammograms for obtaining a calibration graph for Amaranth. Accumulation potential -400mV, Amaranth concentation: Accumulation time, 2 min.
A, 0; B, 1×10^{-8}M; C, 2×10^{-8}M; and D, 3×10^{-8}M.
(Reproduced with permission.)

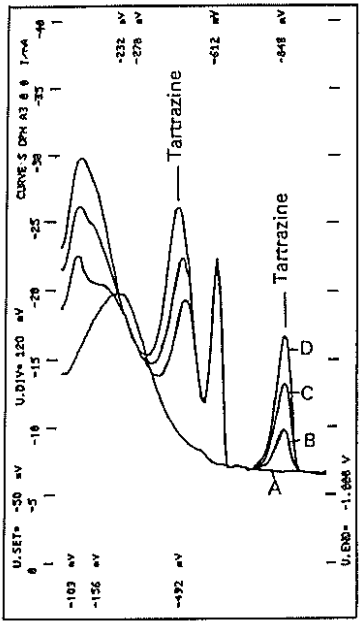

Figure 5
Determination of Tartrazine in a tablet coating. A, Blank; B, Sample solution (with 100 μg mL^{-1} TPPC added); C and D, Standard additions of 2.5×10^{-8}M Tartrazine, respectively.
(Reproduced with permission.)

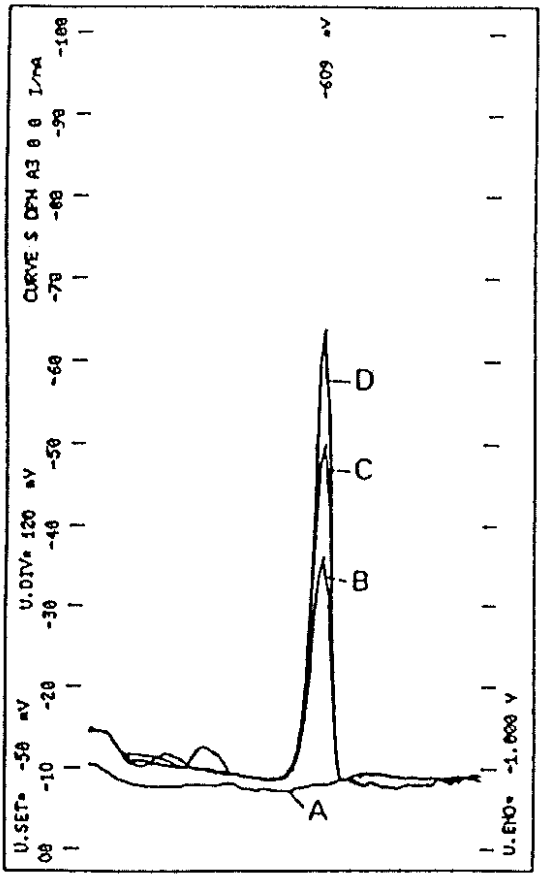

Figure 6 Determination of Amaranth in tablet coating. A, Blank; B, Sample solution; C and D, Standard additions of 2 and 4×10^{-8}M Amaranth respectively. (Reproduced with permission.)

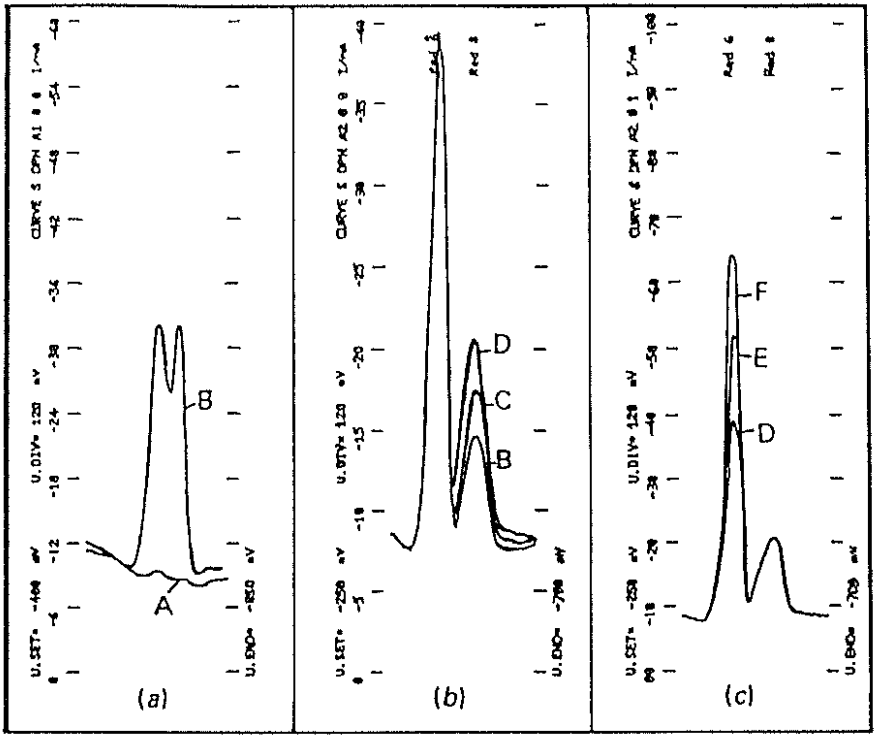

Figure 7 Determination of Lithol Rubine and Lake Red C in a lipstick. Differential pulse adsorptive stripping voltammograms at (a) pH 10; and (b) and (c) pH 7. A, Blank; B, Sample; C and D, Standard additions of 3 and 6×10^{-8}M Lake Red C respectively; E and F, standard additions of 1.5 and 3×10^{-8}M Lithol Rubine respectively. (Reproduced with permission.)

Conclusions:

The advantages of the adsorptive stripping voltammetric analysis are clearly defined. The combination of analyte preconcentration from the sample solution itself along with matrix effect elimination or matrix optimisation (either by dilution or matrix exchange) present a very powerful argument for the use of these techniques for the determination of low levels of a wide variety of pharmaceuticals as well as permitted colouring agents in both the food and cosmetic industries.

The fact that the whole determination is carried out under microprocessor control enables easy instrumental operation while allowing the operator the benefit of custom-designed programming in order to take into account the individuality of the samples themselves.

The increase in sensitivity over the (normal) differential pulse technique (at the Dropping Mercury Electrode, or DME), is between nine- and one hundred-fold depending on the material concerned. Taking the colouring agents as an example the use of longer accumulation times can allow the determination of levels down to 1×10^{-10}M.

Certain agents can also be employed to enhance the stripping signal, such as TPPC. This material can also be used in some cases to effect an extra degree of resolution, as the enhancement effect is only with certain compounds (Colourants, reference 36).

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